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Proceedings of the Eleventh Annual Aquatic Toxicity Workshop:
November 13-15, 1984
Vancouver, British Columbia

Compte rendu des communications du onzième atelier annuel sur la toxicité aquatique:
du 13-15 novembre, 1984
Vancouver (Colombie-Britannique)

D. Leong

Editors/Éditeurs

G.H. Geen and K. L. Woodward

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This document contains abstracts and/or papers from 60 presentations. The material was only subject to minor editorial review.

The Aquatic Toxicity Workshop is achieving international status judging by the number of U.S. and international participants (see Appendix I). The various papers dealt with topics ranging from basic research through analysis of regulatory policies concerning the quality of the aquatic environment. The papers describe work on both the freshwater and marine organisms exposed to a variety of toxicants. This publication may be obtained from:

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Nous tenons à remercier en particulier Mike Halleran de la Fédération de la Faune de la C.B. pour une présentation très intéressante et stimulante lors du banquet.

Ces comptes-rendus furent complétés avec le support de la branche d'Aménagement de l'Habitat des Poissons, département des Pêches et Océans, Ottawa, Ontario.

Ce document contient des résumés et/ou articles provenant de 60 présentations. Le matériel ne fut soumis qu'à des changements éditoriaux mineurs.

L'Atelier en Toxicité Aquatique jouit d'une réputation internationale, à en juger par le nombre de participants provenant des É.U. et de plusieurs autres pays (voir Appendice I). Les articles traitent de sujets allant de la recherche de base à l'analyse de politiques concernant la qualité de l'environnement aquatique. Ils décrivent des travaux portant sur les environnements d'eau douce et d'eau salée, utilisant plusieurs types de substances toxiques, et touchant une variété de plantes et d'animaux.

On peut obtenir une copie de cette publication en écrivant à:

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THE EFFECTS OF MINE WASTES ON AQUATIC SYSTEMS

PAPERS AND ABSTRACTS

PROBLEMS OF METAL BIOACCUMULATION ARISING FROM MINING INDUSTRY DISPOSAL OF TAILING TO THE SEA

Ellis, Derek V. Biology Department, University of Victoria, Victoria, B.C., V8W 2Y2

After more than a decade of field investigations, there is no evidence from trace metals monitoring at the many marine discharging mine sites that bioaccumulation can and does spill over into trophic level biomagnification, and thereby put biological resources and human health at risk. Only a few mine sites have such high bioaccumulation levels that they provide adequate field sites for testing the resource significance of laboratory based toxicology research. The case history results demonstrate a need for site-specific interdisciplinary studies of trace metal bioavailabilty, biolocation at inter- and intracellular levels, correlated pathologies and resource impact.

PROBLÈMES DE BIOACCUMULATION DE MÉTAUX PROVENANT DE DÉCHÊTS D'INDUSTRIES MINIÈRES JETÉS À LA MER

Ellis, Derek V. Biology Department, University of Victoria, Victoria, B.C., V8W 2Y2

Après plus d'une décennie d'études sur le terrain, la surveillance des métaux à l'état de traces à plusieurs sites marins de décharges minières n'a encore fourni aucune évidence que l'accumulation dans les tissus vivants se répande et s'amplifie dans les niveaux trophiques et de par ce fait présente un risque pour les ressources biologiques et pour la santé humaine. Seulement quelques sites miniers ont un haut niveau de bioaccumulation tel à pouvoir servir de site de terrain adêquat pour tester la portée de résultats toxicologiques de laboratoire sur des ressources naturelles. Les résultats d'histoire des cas ont démontré un besoin pour des études interdisciplinaires à des sites spécifiques sur la biodisponibilité de métaux à l'état de traces, sur la biolocation à des niveaux inter- et intracellulaires, sur des corrélations de pathologies, et sur l'impact sur les ressources.

INTRODUCTION

Coastal mines which discharge their mill tailings to the sea, or to landforms from which metal-loaded streams discharge to the sea, are commonly believed to be the source of toxicological damage to marine resources and even public health. There is now a substantial set of relevant monitoring case histories which allow reviews to determine the following:

[1] Is the social concern justified, and have cases of toxicological damage to resources and public health resulted?

[2] Do the sites provide field testing areas to show whether laboratory based toxicological results are relevant to real ecosystem situations, hence to social needs?

[3] Do the sites provide opportunities for novel toxicological research?

MATERIALS AND METHODS

Thirty-two coastal mines, mining areas or smelters are listed in Table 1. Environmental information is available from at least twenty-four of these. Fifteen of the sites have metal loadings data banks from one or more of the receiving ecosystem compartments, i.e. from the discharged effluent, the water column, sediment particulates, sediment interstitial water, plants, herbivores, particulate feeders and carnivores. Of these sites, five provide extensive data banks more or less systematically covering a broad spatial area encompassing reference stations, and over periods of time ranging up to fourteen years. Undoubtedly there are mines with relevant data other than those listed in Table 1, but it is difficult to obtain information about them for various reasons.

The mine sites with the most extensive metal-related data banks are Island Copper Mine on Vancouver Island, and the Kitsault molybdenum mine in northern B.C. Other west coast Canadian mines provide some information, the combined data bank reflects the traditional practice of discharge to the nearby sea in B.C. and, regulatory agency policy since 1970 has been to permit and monitor this traditional practice to determine impact consequences.

Other areas have undergone intensive investigations of particular metal-related ecosystem compartments. They are the Nanisivik Pb-Zn mine on northern Baffin Island, the Black Angel Pb-Zn mine in Greenland, and the Bougainville Copper mine in Papua New Guinea. In each case there has been a need to ensure protection of subsistence resources for native peoples. Also, oyster stocks near abandoned gold mine estuarine waste dumps in Tasmania have been subjected to intensive investigations of metal uptake.

Table 2 provides lists of species which have been subjected to tissue analyses at the intensively investigated mine sites, and shows the elements for which analyses were undertaken, and the years of sampling. Substantial numbers of species have been tested at some sites, notably Island Copper, Kitsault and Nanisivik. At others there has been a concentration of particular species and elements, e.g. Black Angel (mussels and wolf-fish), Jordan River (littleneck clams), Bougainville (fish) and Tasmania (oysters). At Island Copper a wide range of species adopted for tissue monitoring in 1971 has been modified over

Table 1. Marine discharging mines and mining areas.

Mine	Main Product	Environmental Impact Assessment	Introductory References
CANADA			
1) Island Copper	Cu/Mo	Extensive*	Pelletier, 1982 Island Copper, 1984 Waldichuk and Buchanan, 1980
2) Kitsault (closed)	Mo	Extensive*	Burling et al., 1981, 1983 Goyette and Christie, 1983 Amax, 1982
3) Wesfrob (closed)	Fe/Cu	Some*	+
4) Yreka (closed)	Cu	None	+
5) Texada (closed)	Fe	Some	+
6) Jordan River (closed)	Cu	Some*	Ellis and Popham, 1983
7) Brynnor (closed)	Fe	Some, after closure	+
8) Britannia (closed)	Cu	Some*	Ellis and Popham, 1983
9) Polaris	Pb/Zn	Some	+
10) Nanisivik	Pb/Zn	Pre-operational* Some later	+ (chose lake disposal) Thomas and Metikosh, 1984 Fallis, 1982
11) Kitimat (smelter)	Al	Some	Hocking et al., 1980
U.S.A.			
12) Quartz Hill (under development)	Mo	Extensive*	USDA, 1984
GREENLAND			
13) Black Angel	Pb/Zn	Extensive*	+
NORWAY			
14) Fosdalens Bergverk	Fe	Some	+

Table 1 cont...

Mine	Main Product	Environmental Impact Assessment	Introductory References
15) Repparfjord	Cu	Some	+
16) Stjernoy	Fe	Not known	+
17) Ranafjord	Fe	Some	NIVA, 1977
U.K.			
18) Cleveland Potash	Potash	Some	+
19) Hayle Estuary	Sn/Cu	Some, after closure*	+
MEDITERRANEAN			
20) Pechiney	Al	Not known	+
21) Pennaroya	Pb/Zn/Fe	Not known	+ Down and Mill, 1978
RED SEA			
22) Atlantis Deep	Zn/Cu/Ag	Extensive	Mustafa and Amann, 1980 Fletcher and Mustafa, 1980
MALAYSIA/THAILAND			
23) Bhuket/Aokam	Sn	Not known	-
24) Bhuket/Tongkah	Sn	Not known	-
25) Billiton	Sn	Not known	-
26) Ma On Shan	Fe	Not known*	+
PHILLIPINES			
27) Marcopper	Cu	Some*	+
28) Atlas	Cu	Some*	+ Alino, 1984
PAPUA NEW GUINEA			
29) Bougainville	Cu	Extensive*	+ Powell et al., 1981

Table 1 cont...

Mine	Main Product	Environmental Impact Assessment	Introductory References
AUSTRALIA			
30) Yabulu (refinery)	Ni	Some*	+
31) Tasmania (waste dumps)	Au	Some*	Ratkowsky et al., 1974 Ayling, 1974
CHILE			
32) El Salvador	Cu	Some	Castilla, 1983

*Metal data available.

+See Poling, 1982, for bibliography on most mines listed.

Table 2. List of species tested for tissue metal levels at various mine sites.

Mine Sites	Years of Observations
ISLAND COPPER (As, Cd, Cu, Hg, Mo, Zn, Pb)	
Plants	
Fucus distichus, rockweed	1976 annually
Zostera sp., eelgrass	1978 annually
Zooplankton	
total)	1972 annually
euphausids)	1975 annually
Shrimp	1975 annually
Cancer magister, Dungeness crab	1971 annually
Bivalve molluscs	
Mya arenaria, soft-shelled clam	1971 annually
Protothaca staminea, littleneck clam	1971 annually
Macoma irus	1975 annually
Mytilus edulis, blue mussel	1974 annually
Humiliaria kennerlyii (a deep water clam growing on tailings beds)	1977 annually
Saxidomus giganteus, butter clam	1971 annually
Fish	
Many species	1971 annually to 1976
KITSAULT (Cu, Cd, Fe, Zn, As, Mo, Ni, Cr, Pb, Mg, Al)	
Plants	
Fucus distichus, rockweed	1977, 1978, 1981
Shrimp	
Several species (analysed separately)	1978, 1980, 1981
Crab	
Lithodes aequispina	1978, 1980, 1981
Chionoecetes bairdi	1980, 1981
Bivalve molluscs	
Mytilus edulis, blue mussel	1977, 1978, 1981
Yoldia thraciaeformis/montereyenis	1978, 1981
Clinocardium, cockles - 2 species	

Table 2 cont...

Mine Sites	Years of Observations
Fish	1980, 1981
Sole, several species (analysed separately)	
KITIMAT (F)	
Fucus distichus	1976
Ectocarpus sp.	1976
Balanus glandulosa?	1976
Macoma inconspicua	1976
NANISIVIK (As, Zn, Cd, Fe, Cu, Pb, Hg, Mn)	
Mya truncata	1975, 1979, 1982
Boreogadus saida	1974-1975 baseline
Zooplankton	1974-1975 baseline
Fucus vesiculosus	1974, 1976, 1979
Strongylocentrotus droebachiensis	1979
Laminaria solidungula	1976, 1979
Agarum cribrosum	1979
Myoxocephalus quadricorni	1979
Myoxocephalus scorpioides	1979
Leptasterias polaris	1979
Serripes groenlandicus	1975, 1979
Cardium ciliatum	1975, 1979
Holothuria	1979
Palmaria palmata	1976
Astarte boreali	1975
Modiolaria nigra	1975
Hiatella arctica	1975, 1979
Buccinum - 3 spp.	1978
Sipho	1975
BOUGAINVILLE (Cu, Pb, Zn, Cd, Hg, As, Mo)	
Many fish species [8 reported in Powell et al., (1981)]	Several years

Table 2 cont...

Mine Sites	Years of Observations
TASMANIA (Zn, Cd, Cu, Cr, Pb)	
Crassostrea gigas, Pacific oyster	1973
WESFROB (Fe, Pb, Zn, As, Cd, Ni, Hg)	
Bivalve molluscs	
Haliotis kamskatchana, abalone	1971-1974?
Mytilus edulis, mussel	1971-1974?
Hinnites, rock scallop	1971-1974?
+ others intermittently	
JORDAN RIVER (Cu, Zn, Cr, Cd, Pb, Ag)	
Protothaca staminea, littleneck clam	1972-1978
+ others initially	
BRITANNIA BEACH (Cu, Zn, by x-ray energy spectroscopy)	
Mytilus edulis, mussel	1980
Protothaca staminea, littleneck clam	1980
Macoma baltica	1980
Plants	
Fucus, rockweed	1980
Ulva, sea lettuce	1980
Laminaria, kelp	1980
BLACK ANGEL (Zn, Pb, Cu, Cd, Ni, Ag, Fe)	
Fish	
Anarrhicas minor, wolf fish	1973-1977
Platysomatichtys hippoglossoides, halibut	1973, 1974
Mytilus edulis, mussel	1972, 1973, 1976
Fucus spp, rockweed	1972, 1973, 1976

the years, i.e. fish were dropped, bivalve species changed, and some crustacea and marine plants introduced.

RESULTS

Table 3 provides a summary of elevated tissue metal levels detected at the well investigated sites. Some algae and some bivalves show elevations of some metals at some sites. Comments from the originating reports are paraphrased, and the metal data is given in the various formats reported.

DISCUSSION

The range of species tested

At sites where a range of species has been tested such as at Island Copper, Kitsault and Nanisivik, we should consider whether the species are representative samples of the biological communities. Almost certainly they are not, in spite of the number of species tested, and equally it is probably impossible to get such a representative sample without stratifying sampling in some way. Marine benthic fish and plankton communities are highly diverse and variable (for B.C. benthos as an example see Levings et al., 1983 and Ellis, 1969 and 1971, and for arctic benthos see Ellis, 1960). The Island Copper initiative of changing the original species tested reflects a real world situation that only some species are suitable for tissue metal monitoring and toxicological research. These species must be large enough for tissue extraction, abundant, slow moving or sessile for easy collection, consistently available from sampling to sampling, and preferably do not take up inorganic particles with biologically unavailable metal. The species-year list from Nanisivik and Kitsault (Table 2) shows how inconsistent survey collections can be. Some method of sampling species in a relevant and consistent stratification is needed.

From the point of answering the vital question whether there is toxicological damage to resources and public health, the only reasonable stratification is to sample the various ecosystem compartments through which metals will flow. A model of such compartments at which metal determinations and level of impact can be made is given in Table 4. Measures of concentrations and total amounts in the various compartments then allows a bookkeeping approach to metal flow determining how much of the total input has gone into low level sinks, how much is dispersed harmlessly and exported by water currents, plankton or migratory animals, how much is moving up the food network, reducing in concentration as it goes (see Black Angel wolf-fish, Table 3), how much is moving up a food chain and biomagnifying, what is the final compartment sink for such biomagnified metals, at what stage does resource impact occur, and whether public health is at risk.

The ecologist's approach is obviously a complicated, time-consuming and costly procedure. Fortunately there are other ways of achieving equivalent levels of resource protection.

The potential for biomagnification

The reported metal results summarized in Table 3 give no indication that

Table 3. Mines and species with elevated levels of metals in a spatial or temporal pattern suggesting bioaccumulation from mine wastes. Mg/kg dry weight unless otherwise shown.

Mine Sites			Notes
ISLAND COPPER[+]			
Fucus, rockweed	Cu	50-60	)
	Zn	30-100	) May be attached particles
	Cd	4.0-4.5	)
Mytilus edulis, mussel	Cu	2	Consistently higher at loading dock than reference docks (Cu = 1.5) but no trend to increase
	Zn	35	
KITSAULT			
Yoldia	Pb	200-300	1981 increase from 10-50 in 1978
	Cd	30-35	1981 increase from 18-20 in 1978
			Deposit feeding small species
			Not always available
			Highly variable results
			No size related data
WESFROB			
	None		
JORDAN RIVER			
Protothaca staminea, littleneck clam	Cu	20-30	About double reference specimens
BRITANNIA BEACH			
Mytilus edulis, mussel	Cu	645	Viscera
	Zn	607	Gill
Protothaca staminea littleneck clam	Cu	119	Viscera
	Zn	100	Viscera
Macoma baltica	Cu	160	Gill
	Zn	448	Viscera

Table 3 cont...

Mine Sites				Notes
Britannia Beach cont...				
Plants				
Fucus, rockweed	Cu	111		
Ulva, sea lettuce	Cu	345		
	Zn	218		
BLACK ANGEL (Pb is wet weight)				
Fucus, rockweed	Zn	20-100	(1977)	Zn generally increased from 1973 to 1974 after discharge started
	Zn	100-500	(1974)	
	Pb	40-50	(1977)	Pb increased from = 10 in 1973
Mytilus edulis, mussel	Zn	40-70	(1974)	Zn generally increased from 1973 to 1974 after discharge started
	Zn	100-550	(1977)	
	Pb	40-50	(1977)	Pb increased from = 10 in 1973
Wolf-fish liver	Pb	2.5		Predator
KITIMAT				
Ectocarpus	F	317		Higher at 150 m from outfall than at 500 m
Amphipods	F	1168		Higher in amphipods than in barnacles or bivalves (*Macoma*)
NANISIVIK				
Mya truncata	Pb	5.4		No biochemical impact
	Zn	584		No obvious pathologies (histological examination) Exceed maximum levels for marine products
Fucus vesiculosus	Pb	28.3		Increased
	Zn	x 5.6		
	Cd	?		
	As	x 1.4		
Strongylocentrotus droebachiensis	Pb	23.9		Increased
	Zn	x 4.3		
	As	x 1.1		

Table 3 cont...

Mine Sites		Notes
Nanisivik cont...		
Serripes groenlandica	Pb x 19.7 Zn x 2.3 Cd x 1.9 As x 1.7 Hg x 4.7	
Cardium ciliatum	Pb x 1.4 Zn x 1.7 Cd x 2.4 As x 1.7 Hg x 2.2	

+Range approximated from diagrams, 1983 data.

Table 4. Measurement points in the flow of metals through an ecosystem.

Measurement Points	Notes
1) Effluent (tailings)	Total and biologically available metal measures needed for all relevant trace metals
2) Turbidity field	
3) Water column	
4) Sediments	
5) Interstitial water	
6) Whole organisms	Tissues homogenized Includes unmetabolized metals
7) Organism tissues	Metals present may be detoxified (sequestered), e.g. metallothioneins, shells X-ray microanalysis to distinguish between metabolized and unmetabolized metal
8) Organism pathologies	Correlations needed with tissue metal sites; intercellular and intracellular
9) Stock losses (nos. organisms)	Correlations needed with extent of pathologies
10) High trophic levels	Food chain routes need determination May be biomagnification or bioreduction
11) Public health measures (human measures)	If cause for concern

biomagnification occurs at marine discharging mines. However, the lack of indication is in part due to difficulties of appropriate sampling. There are two alternatives to simple metal analyses which can support an appraisal of biomagnification risk.

A resource inventory and appraisal of resource use can show whether the upper level ecosystem compartments of biological resources and humans near the waste receiving area are at the end of an almost closed food chain, from which potentially bioaccumulating metals cannot escape but will be channelled upward. If a proposed receiving area is occupied by a population of fishermen drawing for subsistence on bioaccumulating species, or if commercially fished stocks of predators or suspension feeding shellfish are present, then there are reasons for social concern and action. If the discharge is nevertheless permitted on the grounds of improbable risk, a monitoring programme of the socially important food chain should be required.

A second approach to appraising the likelihood of biomagnifications is use of the Cs-K ratio analysis developed by Young (1982). He showed that in the Californian inland salt lake, the Salton Sea, food chains of phytoplankton to striped mullet, and the detritus feeding polychaete Neanthes succinea to two fish species and then to a top level carnivorous fish, gave high ratio values for fish muscle. Young predicts that the Cs-K ratio is a simple test that, applied to resource species, can indicate the possibility of biomagnification. The ratio needs wider testing as a useful indicator of risk, and the most appropriate monitoring.

Where resources are considered to require monitoring, simple testing of metal levels in tissues is insufficient, since such monitoring does nothing to appraise impact. What is needed is development of studies initiated at Nanisivik in 1982, where a start has been made on relating metal levels to pathology (Thomas and Metikosh, 1984).

Bioavailability of toxins

There is a widespread opinion that animal specimens collected for tissue metal determinations should be depurated of inorganic particles which can bear component or adsorbed trace metals not biologically available to living organisms. Collected algae and eelgrasses at Island Copper mine have also been checked by scanning electron microscopy; it is suspected that inorganic particles can adhere to the rockweed Fucus particularly, and be resistant to cleaning. In this context the problem for field studies is the degree in which inorganic particles lower the precision of the metal analyses, and possibly the accuracy also (depending on what the investigator thinks he or she is investigating, e.g. total, metabolized or sequestered metals). Lack of precision comes from many factors including amount of attached particles, varying concentrations of component and adsorbed metals, the extent to which animals feeding on particle-carrying organisms may be able to digest the metals present and finally, the extent to which depuration and cleaning is incomplete.

The resolution to the problem is to use species which do not require extreme cleaning or depuration procedures, unless there are no alternatives. In this context, slime-producing algae such as the rockweed Fucus, and deposit-feeding bivalve molluscs such as species of the genera Macoma (Reid and

Reid, 1969) and Yoldia (Stasek, 1965 and Drew, 1899) are suspect. Mussels and oysters as accessible shellfish with known ability to bioaccumulate are preferred species, as are benthic feeding resource species such as crustacean shellfish, flatfish and codfish.

CONCLUSIONS

The following tentative answers can now be given to the three questions set in the introduction:

[1] There is very little evidence to date that at marine discharging mines, trace metals do more than bioaccumulate at single trophic levels. If they do flow to higher levels, their concentration reduces and does not biomagnify. In other words, such trace metals appear to be entering low level ecosystem sinks, such as thick layers of tailings deposits, or they disperse at low and harmless concentrations through the ecosystem by currents, plankton or migrants.

[2] Few sites have accumulated trace metals in biological sinks and to levels where contaminated specimens are consistently available to toxicologists for field appraisal of the relevance of their laboratory investigations to social problems. Of these the Black Angel mine in Greenland and the Nanisivik site in Baffin Island appear to provide reasonable opportunities for field investigations.

[3] The important toxicological research needed for ecosystem analysis consists of interdisciplinary studies of trace metal bioavailability, biolocation at inter- and intracellular levels by electron microscopy and x-ray microanalysis, pathology correlations, and resource impact.

In addition, the case history data indicates that improved toxicological impact assessment can be brought about by rational selection of resource species, and a limited range of other species, for tissue metal and pathology measures.

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APPROACHES TO WASTE MANAGEMENT & ENVIRONMENTAL PROTECTION AT WESTMIN RESOURCES LTD., MYRA FALLS OPERATIONS

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Westmin Resources Limited is presently expanding operations at its Western Mines Division from 875 tpd to 2700 tpd.

Western Mines Division is located at Myra Falls in Strathcona Park (a class B park) some 93 km from Campbell River, Vancouver Island.

Mining commenced from this area, by Western Mines Ltd. (now Westmin Resources Limited) in 1965 at a production rate of 750 stpd. This production rate has increased to the present rate of 875 tpd. Three concentrates are produced - zinc, copper and lead - which contain varying amounts of gold, silver and cadmium. These concentrates are trucked to Campbell River where they are shipped from Westmin Resources' dock facilities.

In the latter part of 1979, a new orebody was discovered called the H-W orebody. This orebody is significantly larger than the presently mined areas and will enable production to increase some threefold and also enable production to continue into the next century.

The mine is located in a Provincial Park and also adjacent to Buttle Lake. Major environmental concerns relate to water quality and downstream fisheries protection.

This paper deals with the present developments of the mine and surface facilities, the environmental concerns of the area and the methods and procedures utilized to deal with these concerns.

Specific emphasis is placed on the land disposal of tailings by the subaerial technique, collection and treatment of waste dump leachate, water treatment and sludge disposal.

QUELQUES FAÇONS D'ABORDER LA DISPOSITION DES DÉCHÊTS ET LA PROTECTION DE L'ENVIRONNEMENT À WESTMIN RESOURCES LTD, MYRA FALLS OPERATIONS

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Westmin Resources Ltd. est présentement en train d'élargir ses facilités minières à la succursale Western Mines, de 875 tonnes par jour (tpj) à 2700 tpj.

Western Mines est située à Myra Falls dans le parc de Strathcona (un parc de classe B) à environ 93 km de Campbell River, sur L'île de Vancouver.

L'exploitation minière a commencé dans cette région avec Western Mines Ltd. (maintenant Westmin Resources Ltd.) en 1965 à un taux de production de 750 tpj. Ce taux de production a augmenté jusqu'à un présent taux de 875 tpj. Trois concentrés sont produits - zinc, cuivre et plomb - lesquels contiennent différentes quantités d'or, de cuivre et de cadmium. Ces concentrés sont transportés par camion à Campbell River, d'où ils sont expédiés par bateau à partir des facilités portuaires de Westmin Resources.

Un nouveau gisement, nommé le minerai H-W, fut découvert vers la fin de 1979. Ce gisement est significativement plus large que ceux présentement minés dans la région et il devrait permettre de tripler la production actuelle et aussi d'assurer la production pour le prochain siècle.

La mine est située dans un parc provincial et est adjacente au lac Buttle. Les principales inquiétudes environnementales se rattachent à la qualité de l'eau et à la protection des pêches en aval du lac.

Cet article traite des présents développements de la mine et des facilités de surface, des intérêts environnementaux de la région et des méthodes et procédures utilisées pour tenir compte de ces intérêts.

L'accent est mis sur la disposition des déchêts terrestres par une technique aérienne, sur la collection et le traitement des filtrats de déchêts, sur le traitement des eaux et sur la disposition des vidanges.

AN OVERVIEW OF THE EXISTING AND EXPANDED OPERATIONS

Westmin Resources Limited, currently operates an 875 tpd copper-lead-zinc-mine and mill complex in Strathcona Provincial Park on Vancouver Island, at the south end of Buttle Lake near Myra Falls, B.C. The company also operates a ship/barge loading facility at Tyee Spit approximately 1.6 km north of the community of Campbell River and 93 km northeast of the mine site. An expansion is in progress to increase the mine production rate to 2700 tpd to be completed early 1985. This will involve a new 2700 tpd mill, office-dry, warehouse-shops complex, tailings disposal system, overland conveyor system and development of a new orebody, the H-W. The existing dock facility will also require upgrading to increase its storage and handling capacity.

Present operations of Westmin Resources Limited consist of two underground mines, Lynx and Myra, from which ore averaging 2.05 g/t Au, 72 g/t Ag, 1.0% Cu, 0.9% Pb, 7.7% Zn is transported by car and/or truck to the ore crushing plant and concentrator located immediately adjacent to the Lynx portal. There are also two small but now exhausted open pits associated with each of the Lynx and Myra ore zones and one underground mine which is presently under development: the H-W Mine. Mining in the Lynx and Myra Mines is carried out by cut-and-fill and blasthole stoping methods.

Expanded production will be obtained from the H-W orebody, which is generally a horizontal tabular deposit some 300 to 500 m below the Myra valley floor. Two major types of ore have been identified; polymetallic ore, grading an average 2.85 g/t Au, 90.86 g/t Ag, 2.16% Cu, 1.02% Pb and 9.96% Zn and blend ore, grading 2.13 g/t Au, 17.49 g/t Ag, 2.15% Cu, 0.09% Pb and 3.72% Zn. Access to this orebody is by 6-compartment shaft, measuring 6.56 m x 4.38 m, which was completed to a total depth of some 712 m in April 1983. Based on present knowledge of ore configuration and ground conditions, mining will be by mechanized methods. Ore will be delivered via an ore pass system to a coarse ore bin located above a 1070 x 1220 mm underground jaw crusher. Ore will be delivered after crushing by conveyors from a fine ore bin below the crusher to the automated loading pocket hoisting system for hoisting to surface using a 3.81 m double drum 1200 kw hoist.

Coarse tailings will be used for mine backfill and shortfall will be supplemented with waste rock reclaimed from the surface. Mine water will be presettled underground and pumped to a Water Management System for treatment before release to the natural environment. Ore will be transported by a two flight, rectangular enclosed, elevated, overland conveyor some 1400 m to a new mill located adjacent to the existing facilities. The existing mill employs three-stage crushing, two-stage crushing and differential flotation to produce separate copper, lead and zinc concentrates. Values of gold and silver report to the copper and lead metal sulphides. The new mill, which is now under construction will employ similar circuitry, but has been designed as twin 1350 tpd circuits to enable flexibility of operation.

At the present time, copper and zinc concentrates are hauled 93 km by covered truck to Tyee Spit, 1.6 km north of Campbell River, at which point they are transferred to barge or deep sea vessels for "off-island" shipment from a wharf with berthing capacity for 10,000 DWT ships. The low volume lead concentrates are trucked to the railhead at Courtenay. There will be no change to this arrangement for the mine expansion.

Process water for the existing plant operations is obtained from recycling water from underground, internal recycle, Watertank creek and from the hydroelectric power plant tail race. Water for the new mill facilities will be obtained from these same sources and by recycle of supernatant from the mine water treatment system.

Power requirements for the existing operations average 2.17 MW and peak loads reach 4.8 MW. These are presently supplied by a hydroelectric power plant on Tennent creek, penstocked from reservoir facilities on Tennent Lake and supplemented by 8 diesel electric generating units. The new facilities will require an additional 10 MW of power. Increasing the local hydroelectric generation by expanding the existing Tennent Lake capacity and developing the hydroelectric potential of Thelwood Creek was proven to be a more viable and more economical alternative than connection to the B.C. Hydro grid system, which would require bringing in a 35 km overhead line. This alternative also provides potential for downstream fisheries enhancement through control of low and high flows.

The majority of waste rock and overburden from the existing operations was generated between February 1966 and December 1975 during the development and mining of Lynx open pit. Much of the overburden was used in road construction, but the waste rock has been stockpiled adjacent to, and within, the open pit. Much smaller volumes of waste rock are generated by underground mining and are generally stockpiled near the mine access portals. Throughout the life of the mine a large portion of the underground waste rock has been utilized as construction material in building treatment pond berms and in road bed maintenance.

Tailings from the existing mill, which represent approximately 70% of the total mill effluent and contain all the solid waste, are sized by cyclone, and approximately 50% of this amount is returned underground as backfill. The remaining 50% (minus 200 Mesh) is piped as slurry and deep-lake disposed, together with a flocculant, to Buttle Lake, some 3.5 km distance. The tailings from the proposed new mill will be cycloned in a sizing plant for recovery of mine backfill. It is estimated that 40% will be recoverable from expanded production.

Prior to expansion, Lynx Mine water was directed to three settling ponds in series for solids removal. The decant from the last of these was then released to Myra Creek. Myra Mine water was treated in a single large exfiltration pond and the exfiltrate released to Myra Creek.

ENVIRONMENTAL CONCERNS WITH EXISTING OPERATIONS

In early 1980, the Government of British Columbia and Westmin Resources Limited were alerted, by a report released by the B.C. Waste Management Branch, to a statistical analysis of Buttle Lake water which showed that there had been an increasing trend in zinc between 1972 and 1979, particularly with respect to depth. Since the report did not examine tributary waters, the source of the increased zinc content of the lake was not identified.

Westmin undertook to quantify the possible losses of heavy metals from the tailings to the lake in mid-1980 by analyzing core samples of the deposited tailings mass and comparing these to the historical tailings assays for the period the tailings were deposited. However, the results from these studies were found to be equivocal and held in abeyance as being inconclusive.

This study was followed up by several laboratory leaching tests by Government agencies and all of the tests performed indicated that metals could be leached from the tailings. However, all the laboratory tests were conducted with either activation by stirring, aeration or, as in one case, with nitric acid. None of the tests simulated the conditions as found at the bottom of Buttle Lake. Nevertheless, it was rapidly concluded, and generally believed by most people, that the tailings discharge to Buttle Lake was the primary source of the increasing metal levels in the lake. During this period (early 1981) the Provincial Government came under considerable pressure to follow-up on the report findings and requested that Westmin initiate investigations into two areas of primary concern:

a) the source or sources of metal loadings to Buttle Lake.
b) alternate methods of tailings disposal other than deep lake disposal.

Westmin Resources Limited responded to the Government's first request by establishing an independent monitoring committee, funded by Westmin, to conduct the necessary studies.

With respect to the Provincial Government's second request, Westmin commissioned three separate engineering firms to examine the possibility of disposing tailings on-land. From the three submissions, a total of seventeen potential disposal sites were identified, covering 6 disposal techniques. Of the various proposals submitted, the sub-aerial technique was singled out as the preferred alternative for use at the Myra Falls site.

The Monitoring Committee commenced the first phase of their studies in June and July of 1981 which consisted of detailed chemical sampling of the main tributaries to Buttle Lake. The study findings were released in September 1981. Based upon a simplistic model of Buttle Lake it was determined that approximately 90% of the zinc loadings to the lake originated with one tributary, namely Myra Creek, which passed through the area of Westmin's mining operations.

Of the zinc loadings in Myra Creek in those two months, only 5% could be attributable to surface drainages entering the creek and these were primarily in the area of mining operations. The most notable sources were drainage waters from the mined out Lynx Open Pit waste rock piles and the Lynx Mine water treatment ponds. It was assumed that the remaining source was groundwater flow to Myra Creek.

As an immediate approach, Westmin undertook a number of environmental improvements in mid to late 1981 to eliminate or reduce these sources, as follows:

a) Added lime to the Lynx pond system to improve metal removal.

b) Removed the alkaline-chlorination plant effluent from the tailings outfall and rerouted this effluent to the Lynx system for additional metal removal.
c) Increased the capacity of the Lynx ponds to accommodate improved precipitate formation and increased flows.
d) Constructed a surface and groundwater diversion channel above the Lynx open pit and waste rock dumps to divert those waters, which had previously passed through the open pit and waste dumps, to Myra Creek downstream of mining operations.
e) Initiated the recycle of the Lynx Mine 8 Level water to the mill.

These initiatives were later shown by subsequent monitoring to have resulted in some immediate benefits to downstream water quality. For example:

a) There was ten-fold improvement in the decant quality of the Lynx Mine water treatment system.
b) The volume of surface and subsurface water emanating from the Lynx open pit and waste rock dumps was reduced significantly.
c) The quality of the tailings discharge to Buttle Lake improved significantly.

Since a significant proportion of the metal loadings to Myra Creek were still not identified the Monitoring Committee recommended a second phase of study which included a groundwater component to confirm suspected sources and loading estimates over a one year period. The Phase II program was initiated in November 1981 and continued through to June of 1982. In addition, the Monitoring Committee recommended a limnology investigation of the south basin of Buttle Lake and a parallel *in situ* evaluation of the lake tailings to define the actual contribution of metals originating with the tailings. The lake limnology and *in situ* tailings studies were completed in January and June 1982, respectively.

The lake limnology studies employed a series of profiling techniques to segregate the various inputs within the lake itself during isothermal conditions and to selectively sample these inputs, particularly the water overlaying the tailings, for dissolved metal content. The water quality data confirmed the presence of tailings below 30 m, extending fan-shaped from the tailings raft in a northward direction. However, dissolved metals decreased with depth, the opposite effect expected if metals were dissolving at the tailings mass/water interface.

The *in situ* tailings studies were far more sophisticated and entailed the collection of undisturbed and unexposed core samples from the lake bottom in air-tight tubes. The tubes were sequentially extruded and stored, after being centrifuged under a nitrogen atmosphere to prevent oxidation of the separated sediments and tailings pore waters. These waters were analyzed for dissolved metals and pH. From the results it was determined that the deposited tailings interstitial water, or pore water, was more alkaline and contained significantly lower levels of metals than the overlying lake waters. Further, there was no gradient observed in either pH or metal content with respect to vertical distribution in the tailings. As a result, it was concluded that the tailings in Buttle Lake were non-reactive and that no significant chemical reactivity (leaching) had been releasing these metals from the tailings to the lake water.

From observations during the study, it was also suggested that the natural deposition of organic-rich river sediments on top of the tailings should eventually provide an "anoxic-cover" which would inhibit and eventually prevent metal release that might occur from the tailings veneer after deposition ceases.

The Monitoring Committee Phase II program, which was terminated in June of 1982, confirmed that the major contributor of metals to Buttle Lake was Myra Creek. These loadings originated primarily with the waste rock dump seepage (up to 31% of total zinc, 45% total copper, 71% total cadmium) and with the Lynx open pit drainage (up to 12% total zinc, 13.1% total copper, and 9.8% total cadmium). Minor loadings (between 0.5 and 2.0%) of all metals originated with the Lynx Mine water treatment ponds. The groundwater studies confirmed that the unaccounted for loadings of zinc to Myra Creek primarily originated with contaminated groundwaters in the vicinity of Lynx No. 1 waste rock dump. It was found that precipitation and uphill runoff infiltrates the waste rock piles, picks up metals which have been liberated by the microbiological leaching of the high pyrite host rock and carries the metals as the infiltrating water moves toward the water table. Upon reaching the water table the groundwater moves toward and through the gravels and sands to discharge to Myra Creek some distance downstream.

It was found that metal concentrations generally decrease with increasing distance from the waste rock piles and increased from west to east along the flood plain. Groundwaters from the south side of Myra Creek were found to be relatively uncontaminated. Groundwater from below the sands and gravels provide a small amount of recharge to the upper layers but no significant amount of metals.

GOVERNMENT APPROVAL AND METAL MINE DEVELOPMENT PROCEDURES

In British Columbia all new, expanded or re-opened mining developments must first obtain the sanction of the Provincial Environmental Land Use Committee (ELUC), a nine member cabinet committee, before obtaining the necessary licenses, permits and approvals to proceed. To obtain government "Approval-in-Principle", proponents of mine developments must submit detailed socio-economic and environmental impact assessments to the Metal Mine Development Steering Committee (MMSC), a technical advisory committee to ELUC, for review and evaluation.

For large developments, a socio-economic and environmental impact assessment usually entails the preparation of a Stage 1 Report which provides a preliminary description of socio-economic and environmental resources, a preliminary mine plan outlining the basic development and options, tentative impacts to the community and environment and, areas requiring further work to resolve those impacts. The Stage 1 is submitted to the MMSC for review and a compendium of intergovernmental comments is returned to the proponent for addressing in the Stage 2 Report.

The Stage 2 Report is to include a detailed description of the areas, socio-economic and environmental resources, a detailed mine plan, a description of impacts that are to be expected from the development and, a description of the facilities and methods that would be employed to mitigate or eliminate those impacts.

"Approval-in-Principle" is never granted without conditions. Once "Approval-in-Principle" is received, applications for individual permits, licenses and approvals can be filed.

Along with the Stage 1 and 2 Reports, the MMSC regards a concurrent Public Participation and Information Program during the planning and development process, a corporate responsibility to ensure that government approval is not granted in isolation of, and in opposition to, the wishes and concerns of the community affected. The results of the public review process are of special interest to the MMSC and can weigh heavily in their attitude to a proposed development and possible need for public hearings.

The acquisition of the various necessary permits, licenses and approvals, also known as Stage 3, for each aspect of the development can run concurrently, for the most part, with construction. Some major permits, such as those for hydro-electric developments, tailings dams and reclamation are lengthy, involved procedures requiring the submission of detailed plans, supporting engineering reports, advanced fee payments, advertising and follow-up meetings.

Pursuant to "Procedures for Obtaining Approval of Metal Mine Development (April 1979)", Westmin Resources Limited submitted its Stage 1 Report to the British Columbia Metal Mines Steering Committee on December 1, 1981 for government and public review. The Stage 1 Report provided an overview of the existing operations and facilities, conceptual plans for the expansion, a preliminary inventory of existing environmental and socio-economic conditions, initial environmental and socio-economic impact assessment and, an outline of areas requiring further study in order to finalize the foregoing. The Stage 1 Report was accepted by the Provincial Government on January 14, 1982, and the B.C. Metal Mines Steering Committee returned their "Detailed Government Review Comments" to Westmin Resources Limited on April 5, 1982 as a guide to completing the Stage 2 Submission, but included was the condition that concerns with water quality had to be resolved before Stage 2, "Approval-in-Principle", could be granted.

RESOLUTION OF EXISTING ENVIRONMENTAL CONCERNS

The planning and engineering of a system to collect and treat the surface and groundwaters emanating from the Lynx open pit and waste rock piles required considerable pre-planning in order that the proposed resolution of the existing environmental matters would compliment, rather than hinder, the future expansion planning that was taking place at the same time. For example, areas designated for tailings disposal could not be utilized for waste water treatment. Sizing of treatment works and the type of treatment technology proposed would have to accommodate and be compatible with anticipated future effluent flows and treatment requirements. Scheduling was also critical in that the installation of new treatment systems would have to cope with the existing operations and yet dovetail with the expansion planning.

The obvious solution to mitigate this migration of contaminants to the creek consisted of two elements; the first was to reduce the infiltration of surface run-off into the waste pile; and the second to intercept the reduced seepage from the waste pile before it reached the creek.

The first element to reduce infiltration into the waste pile had already been completed in 1981 by constructing a ditch along the side of the hill immediately above the top of the waste piles to intercept and divert all surface water runoff from above, away from the waste piles. In addition, the filtration of precipitation falling directly into the waste piles was reduced by shaping the surfaces of the waste piles to promote runoff.

The site for the conceptual design for tailings disposal was located between the main waste rock pile and Myra Creek. One of the key elements of the design was that the tailings be placed by the sub-aerial technique directly onto the tailings pile which would be fully drained and consolidated. Supernatant liquid from the tailings surface would be decanted continuously, collected and recycled to the mill or water treatment facility. Included in the design was a sub-surface interceptor drain between Myra Creek and the perimeter of the tailings facility which would enable the natural phreatic surface to be drawn down below the water level in the creek and so induce a backflow from the creek to the drain. This arrangement would positively intercept all seepage from the tailings and prevent it from flowing into the creek. Water recovered from the ditch would be pumped to a water treatment facility.

After the design was submitted it became apparent that the interceptor drain would also intercept seepage emanating from the water rock piles and thereby mitigate the migration of heavy metals into Myra Creek and Buttle Lake.

Field investigations to determine the groundwater flow regime and water quality between the waste piles and Myra Creek were carried out during 1981 and 1982. These investigations included:

- drilling bore holes to determine the stratigraphy of the area;
- pump tests to determine the hydraulic conductivity of the strata;
- sampling of the groundwater to determine the pH, conductivity and water quality of the groundwater;
- installation of peizometers to determine the direction of groundwater flow and;
- construction and operation of a test section of interceptor drain 60 m long to demonstrate the feasibility of intercepting the contaminated seepage before it enters Myra Creek.

The results of the investigations showed that it would be possible to intercept a significant proportion of the contaminants by constructing a subsurface interceptor drain between the waste piles and the creek. The design objective of the drain is to draw the phreatic surface down at the location of the drain sufficient to create a reverse flow condition from the creek into the drain. This would effectively prevent the contaminants from reaching the creek.

The interceptor drain and tailings storage facility was designed to be constructed in two phases, the first being to accommodate the present operation with a second phase extension to accommodate the future expansion program.

The first phase of the interceptor drain, comprising approximately 900 m of drain, was constructed during the summer of 1982 and was commissioned on September 8.

Water from the drain is collected in a sump at the downstream terminus and is pumped to the treatment facilities by 2 vertical turbine pumps having a combined capacity of 200 litres per second.

The water treatment system was constructed primarily on the south side of Myra Creek but includes a surge basin on the north side. Lynx open pit water, including discharges from Lynx 5 and 6 Levels, is combined with the tailings disposal area surface waters and groundwaters and is gravity-fed from the north side surge basin to the first of 5 ponds located on the south side of Myra Creek. The first pond is also a sealed flood stabilization pond and now also receives waste water from the Myra Mine, the H-W Mine and H-W area clarified sewage treatment plant effluent. Flows are then divided into two roughly equal portions at a splitter tank. These two flows are conveyed to parallel scavenger ponds, followed by parallel polishing ponds. The final decants are then recombined before release to the natural environment.

In order to be able to optimize the interception of contaminants and keep pumping costs to a minimum, the sub-surface interceptor drain can be operated in a manner to selectively increase the flow into the system in areas containing heavy contamination and to selectively reduce a flow into the system in areas which contain little contamination or are free of it.

This selective operation of the drain has been achieved by dividing it into discrete lengths ranging from 30 to 100 m in length. The filters and perforated drain pipes which intercept the groundwater flow in each section terminate in a manhole containing an adjustable weir which divides the manhole into two sections and enables the phreatic surface along the section draining into the manhole to be controlled. The water spilling over the weir is decanted into a collector pipe which connects all the sections and conveys it to the pumping sumps at the downstream end of the drain.

Piezometers were installed at regular intervals on either side of the drain and these are used to monitor the level of contamination and phreatic surface of the groundwater in the vicinity of each section.

The Monitoring Committee was requested to undertake the evaluation of the surface and groundwater collection system as Phase III of their studies. Monitoring was initiated at the beginning of September 1982. This included monitoring of between 12 to 16 piezometers on either side of the underdrain, the underdrain waters, the treatment pond supernatant or decant and several locations in Myra Creek along the length of the project area.

On the basis of the total zinc inflows and of outflow measurements at the discharge, the efficiency of the water treatment system has been consistently above 93% and usually greater than 99% since the commencement of monitoring. Efficiencies of less than 98% were the result of high flows and heavy rainfall or a combination of both.

Nevertheless, the final effluent quality of the treatment system has, on a consistent basis, been well within the total zinc limits of 1.0 mg/L specified by the Waste Management Permit.

Total zinc loadings for Myra Creek for the period prior to the installation and operation of the surface and subsurface water collection system were compared to similar data obtained since the implementation of the collection and treatment system. Lines of best fit for both sets of data calculated by the least squares technique, in all cases, demonstrated that for comparable flows, the zinc loadings were reduced by about 75% of their original values.

The total zinc in Myra Creek would have been in the range of 220 to 1100 kg/d if the collection and treatment system was not operating. The actual recorded values ranged from 34 to 396 kg/d and the overall efficiency of zinc removal and treatment after modifications were completed approached 80%. The removal of zinc from the creek at the groundwater pumphouse was more effective. At this location, the actual recorded values ranged from 11 to 158 kg/d and, the overall efficiency of zinc removal and treatment after modifications were completed approached 90%. The remaining zinc loadings to Myra Creek below the pumphouse originate primarily with pyritic waste contained within the tailings line road.

Over the period since the collection and treatment system has been operational, there has also been a dramatic improvement in the water quality of Buttle Lake. Results of monitoring undertaken by not only Westmin, but also the Provincial and Federal Government agencies, confirm that metal loadings in Buttle Lake have steadily declined, and zinc concentrations at the outlet of Buttle Lake have been reduced by 70% of the levels recorded in 1980 and 1981 and are now at a level equivalent to those recorded in the early 1970's. It is expected that this trend will continue.

The collection and treatment system, effective as they were, produced such vast amounts of pure hydroxide precipitates, greatly aggravated by the formation of hydrated aluminum hydroxide, that a solution to sludge disposal had to be found.

Whereas, sludge from the Lynx treatment system is easily settled and can be disposed of by conventional earth moving equipment to secure land disposal sites with the waste rock dumps and behind the surface and groundwater collection system, the Myra ponds sludge because of its purity is very floccy, thixotropic and settles to a density of only 1% solids. As such it consumes a large portion of the settling capacity thus over time, reduces the ponds efficiency.

Consequently, continuous de-sludging facilities, consisting of a submersible Flyght pump suspended from a movable barge system, were installed. Sludge is pumped by a 6" victaulic line to injection trenches or injection wells excavated (drilled) atop the waste rock dumps. Here the high pH waters are allowed to percolate down through the waste rock where they can effect the micro-environment of the leaching bacteria and thus the leaching process.

Isolation of sludge in the ponds is greatly assisted by the use of fabrine, woven Polyethylene curtains weighted by 1-1/2 wire rope and suspended from 8" x 8" timbers. These curtains are also utilized to maximize flow through the ponds or divide the ponds into cells where sludge is retained and supernatant is decanted.

Injecting the high pH sludge into the waste dumps, in effect, makes use of any unused lime from the water treatment system and is an effective way of applying the lime to active internal parts of the dump. Its effectiveness has been clearly seen in the quality of groundwater emanating from the waste rock dumps and intercepted by the groundwater collection system. Over 5 months of monitoring data from 2 access chambers and from the terminal wet well, clearly show a gradual but definite trend in declining zinc concentration and increasing pH.

At this time, the major environmental issues were resolved. The Stage 2 Submission, consisting of 6 volumes in 9 books and included a refined project plan for the proposed mine expansion and wharf modifications, a detailed account of the existing environmental and socio-economic conditions and definitive environmental and socio-economic impact assessment, was submitted to ELUC in August 1982. Also included were a detailed Reclamation Plan and proposals for on-going monitoring and further study.

Government "Approval-in-Principle" was granted on March 11, 1983 after receiving sufficient evidence that water quality improvements were well underway. Board of Director approval was granted March 29, 1983 following the acceptance of a favourable feasibility study.

FUTURE TAILINGS DISPOSAL

Beginning in mid-1984, deposition of tailings from the existing operations into Buttle Lake will cease and will be stored on land. The coarse fraction will continue to be used from underground backfill and the fine fraction will be stored.

The design of the storage facility is integrated with the interception drain and the centre line of the initial starter embankment will be directly over the centre line of the interceptor drain described in the previous section. This means that all seepage of liquids from the deposited tailings will be intercepted and treated in the same manner as the contaminated leachate from the waste piles.

The storage facility will be developed in 2 stages. The first stage will be designed to accommodate the present ore process rate of 875 tpd and in the second stage, the facility will be enlarged when the expansion of the mine increases ore production to 2700 tpd.

The fundamental environmental objectives in the design of the tailings storage facility are:

- Permanent, secure and total confinement of all solid waste material.
- Control and removal of all contaminated waste liquids and strict monitoring of the natural groundwater regime to ensure that seepage of contaminants from the facility is reduced to negligible quantities. In addition, control of existing contaminated groundwater flows is a major objective.
- Permanent facilities to monitor all aspects of the performance of the tailings storage facility including facilities to sample seepage water and to intercept, recover and treat this water, if necessary.

- A decommissioning program which will ensure the long term security of the storage facility and yet require only minimal surveillance and maintenance.
- Minimal aesthetic and environmental impact on the surrounding area.

The fundamental engineering objectives in the design are:

- The general location of the site should be such that all surface runoff from outside the boundaries of the site is minimal and can be permanently diverted away from or around the site.
- The bottom of the confined waste materials should be located above and isolated from the natural groundwater table and have positive underdrainage systems which ensure that vertical seepage from the deposited waste materials can be intercepted and collected for recycling or treatment to the maximum practicable extent.
- All construction materials used in the outer embankments, filters and surface seals should be resistant to chemical attack from the contained materials and have permanent durability characteristics.
- All embankments should be designed and constructed to withstand erosion and have adequate factors of safety for all loading conditions, including dynamic loading resulting from seismic activity.
- The method of deposition of the solid waste materials should be such that they are at least drained sufficiently to reduce the retained mass from a plastic to a solid state. Preferably this drainage should be such that all free draining liquid is removed to the extent that seepage discharge is completely eliminated by the time the project is decommissioned.
- A final seal will be constructed over the entire surface of this facility where the mine is decommissioned which will ensure that infiltration of precipitation and recharge of the pore spaces in the stored solids is eliminated.

A central feature of the design for achieving the above objectives is to obtain a stable, partially saturated, drained and consolidated deposit of tailings such that seepage discharge to the environment will be reduced to negligible proportions during operation and, will be virtually eliminated by the time the operation is decommissioned.

This can be achieved by developing a system in which the liquid fraction of tailings slurry is reduced after deposition by an amount sufficient to produce a partially saturated condition in the deposited solids. Due to the fine grading of the material, this partially saturated condition develops the inherent capillarity in the pore spaces and induces high negative pore pressures which permanently retain any residual moisture in the material. Such a partially saturated deposit is physically incapable of yielding any seepage, provided the negative pore pressures are not dissipated by recharge of the pore spaces with additional moisture from an external source, or, resaturation due to additional loading does not occur. The design of this facility integrates a deposition strategy based on the sub-aerial technique with a comprehensive underdrainage system. Details of the tailings deposition strategy are described more fully later on.

The facility will be located on the north side of Myra Creek and will be constructed against the existing waste rock dump on the north side of the valley, which could ultimately be completely covered. In order to provide sufficient area for the storage facility in a single location when operations are expanded, Myra Creek will require diversion along the south side of the valley over a length of approximately 1 km.

Tailings deposition will be carried out from a starter embankment constructed adjacent to Myra Creek along the length of the storage area. The tailings will be deposited using the sub-aerial technique and will be managed in a manner such that the surface always slopes away from the embankment and towards the waste rock dump on the north side of the valley. A sloping filter will be installed against the rock dump and this, together with a decant to cope with large storm runoffs, will continuously remove water from the tailings surface.

In the sub-aerial technique the tailings slurry is discharged onto a section of gently sloping beach of previously deposited tailings by means of distribution spray bars located along the upper edge of the beach. After it leaves the spray bars, the slurry flows gently over the sloping beach and forms a uniform layer. The slope of the beach and the thickness of the layer formed are functions of the characteristics of the slurry and, experience to date on the pilot test area indicates a typical slope of 1 in 100 will result for these tailings.

Once the section of beach has been covered with the slurry, the discharge is moved to another section of beach and the newly deposited layer is left to settle, drain and bleed. During this process some segregation will occur within the layer, with the finer fractions concentrating at the surface. The liquid released to the surface by the bleeding flows over the sloping surface to the bottom end of the beach to the sloping filter or decant system. Because of the relatively flat slope of the beach the flowing liquid does not generate enough velocity to disturb the solid particles and the supernatant liquid is generally clear and free of suspended solids. In some cases, the scrubbing action of the slow laminar flow over the relatively rough surface helps to remove suspended solids from the liquids.

Drainage of liquid from the newly placed layer also occurs during deposition and continues while settling and bleeding is taking place. Drainage will be induced from the bottom of the layer by suction pressures in the underlying partially saturated layers and will either be absorbed, or, if the underlying layers approach full saturation, drain through the deposit to the underlying underdrainage system. Continuous movement of water through the deposit can only be sustained if the surface is kept in a fully submerged condition.

The time required for settlement, drainage and bleeding to be completed is a function of the solids content of the slurry and the physical characteristics of the tailings solids and, in the case of these tailings, will take up to 4 days.

After the bleeding has ceased, the layer is left exposed and suction pressures are induced as a result of drainage and moisture which is removed by evaporation. These suction pressures induce consolidation, particularly during the summer months when the evaporation is high. The amount of consolidation that can be attained by drying shrinkage is governed by the characteristics of the solids and, in general, finer materials will consolidate to a greater extent than coarser materials. By minimizing the surface area of the tailings covered by the supernatant pond, the amount of moisture lost from the tailings material is maximized, thereby inducing consolidation, in contrast to conventionally placed subaqueous tailings in which evaporative losses generally only offset precipitation input into the pond.

An important characteristic resulting from the sub-aerial technique is the particle segregation which occurs during the settling of each layer. This produces an anisotropic condition in which the vertical coefficient of permeability can be up to two orders of magnitude less than the horizontal coefficient of permeability. This condition reduces the infiltration of precipitation into the deposit and helps maintain the partially saturated conditions in underlying layers. Consequently, the major portion of precipitation on the tailings beach will be shed immediately and recycled to the treatment pond.

After tailings have been allowed to drain, suction pressures inherent in the fine grained material will retain residual moisture below a specific saturation level provided no additional water is permitted to enter the tailings. If the saturation level of the tailings is further reduced by air drying, the tailings will absorb and retain additional moisture until this critical saturation level is reached.

The drained and consolidated condition of the deposited tailings will greatly facilitate decommissioning and reclamation. When mining operations cease, it will be possible to immediately construct a permanent seal over the facility. Unique features of the design are that at full storage capacity and at any time during deposition, no excess pore pressures exist within the tailings mass. The tailings material will be consolidated to densities over and above those that could be achieved by physical loading and dewatering of conventionally place tailings. Settlements during construction of the final seal will be immediate and will be a function of the compressibility of the consolidated tailings only. Once this final seal has been constructed, a condition of no further seepage from the facility can be guaranteed.

It is anticipated that deposition of tailings using the sub-aerial technique will be possible at Myra Falls for at least 9 months of the year. During the remaining winter months, when there is likely to be a significant accumulation of snow on the tailings surface, or periods when ambient temperatures may not allow operation of the spray bars, deposition will be carried out by conventional open ended discharge over a selected portion of the beach.

The thickener layer deposited over part of the tailings beach during the winter period will be left exposed during the ensuing spring and summer to thaw, settle, drain and consolidate, before any further tailings are placed over it. The area selected for winter deposition will be changed each year in order to distribute the thicker layers throughout the facility. This strategy will eliminate the possibility of any large, long term differential consolidation and will prevent the formation of lenses of frozen material being trapped in the tailings.

The bulk tailings from the mill are classified by cyclone to separate the coarse fraction, which is used for cemented fill underground, from the fine fraction, which will be stored in the facility.

Before the design could be carried out, it was necessary to carry out a series of laboratory tests on the tailings material to determine the physical characteristics of the material and the suitability for deposition using the sub-aerial technique.

In addition to the laboratory tests, a pilot scheme was set up at the mine; tailings have been deposited since June 1981 and will continue to be deposited until spring 1983. The deposition area is approximately 30 m^2 and is divided into 3 beach areas. In situ tests carried out on the tailings have included density and moisture content and undisturbed samples have been tested in the laboratory to determine the consolidation, shear strength, permeability and liquefaction parameters of the deposited materials.

The average particle size of the fine fraction of the tailings is 65 microns (±60 mesh).

The results of the tests carried on in the laboratory and on the material deposited in the pilot scheme show that, despite the fine grading, the tailings are well suited to deposition by the sub-aerial technique.

The average density that can be achieved in the drained deposits is approximately 40% greater than that which would be achieved by a conventional wet disposal system. This reduces the capacity of the storage facility considerably with significant cost savings being effected.

The strength parameters of the tailings material after deposition are such that the tailings can be utilized to construct the major portion of the structural elements of the facility thus reducing the cost of the facility significantly.

The vertical coefficient of permeability of the deposited tailing is less than 10^{-8} m/sec and this, coupled with the sloping beaches, results in more than 95% of all precipitation being shed and decanted from the surface of the tailings, thus maintaining a stable, partially saturated deposit which does not yield any significant seepage once the bottom of the storage area is covered with tailings.

The mining property at Myra Falls is located in a relatively active seismic area and is close to the epicentre of Campbell River's earthquake of magnitude 7.3 that occurred in 1946. Seismic stability of the tailings storage facility is, therefore, a major consideration in the overall design. Significant aspects of the design are the stability of the overburden materials forming the foundations of the tailings facility and the seismic stability of the tailings material itself.

It is generally accepted that the majority of earthquakes in Western Canada are associated with motion between three major tectonic units: the Pacific, the

Juan de Fuca and the North American plates. This motion results in a concentration of earthquakes off-shore from Vancouver Island and a random distribution of earthquakes in the vicinity of the site, which is located on the North America Plate. Design values of peak horizontal acceleration for the site have been provided by the Pacific Geoscience Centre as follows:

Return Period (yrs)	Peak Horizontal Acceleration (% g)
100	17.8
200	28.9
475	51.3

For consideration of stability of the overburden materials, the 475-year design earthquake has been used giving a 10% probability of exceedence in 50 years. The 200-year design earthquake was used for consideration of tailings stability during the operating phase of the mine, with a 9.5% probability of exceedence in 20 years and 475-year design earthquake for the long term stability of the tailings embankment.

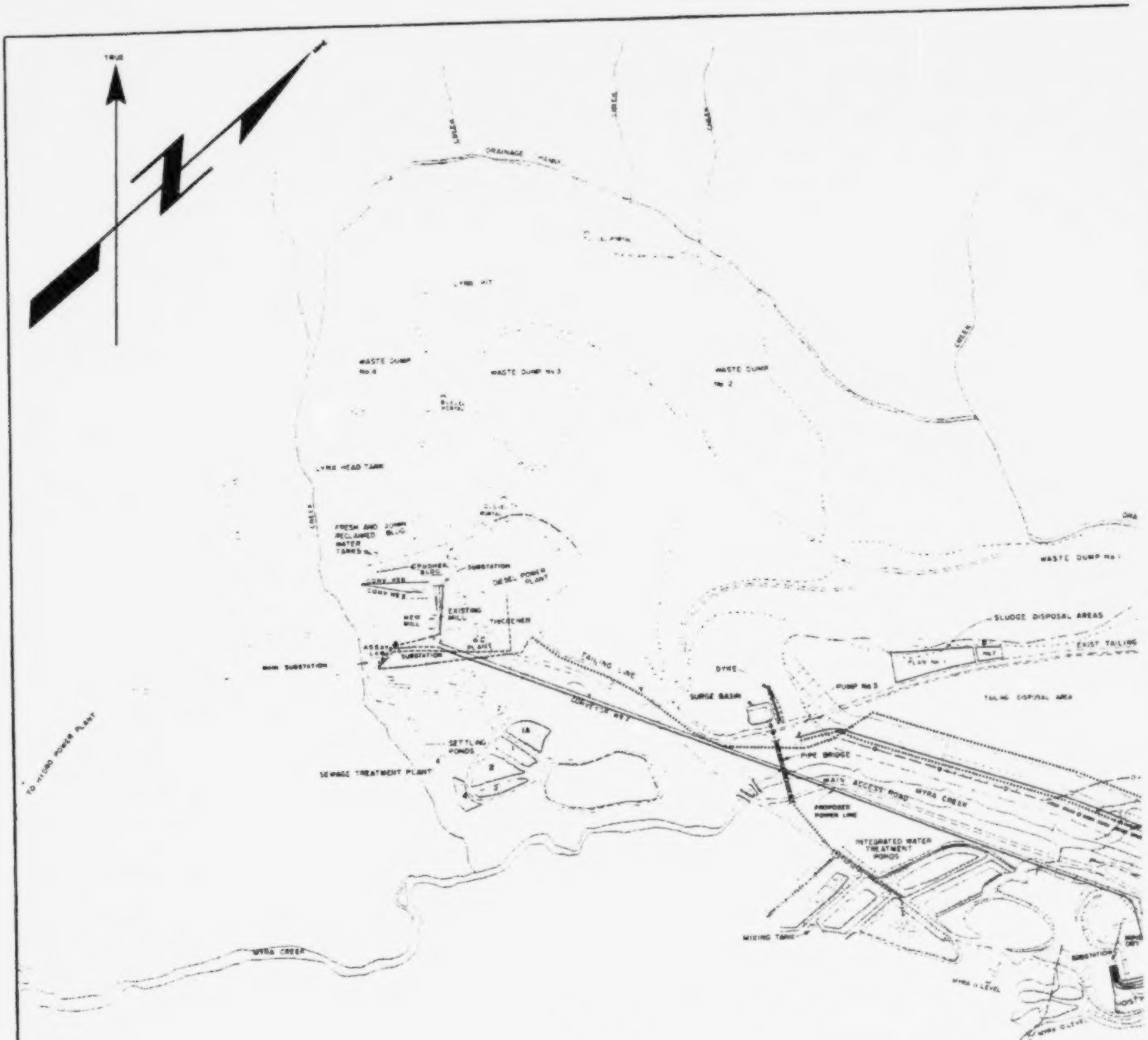
The stability of the overburden materials in Myra Creek valley has been assessed on the basis of the values of resistance to penetration obtained from an extensive overburden drilling program. In general, the overburden materials are very dense, angular sands and gravels which will provide a liquefaction. Some isolated areas of lower density materials were identified and, with correct engineering treatment, any adverse effects of such areas can be readily overcome.

The stability of the tailings material has been assessed on the basis of the measured liquefaction resistance of the material and the pre- and post-liquefaction strength characteristics. A program of cyclic triaxial testing of the tailings material was carried out at the University of British Columbia. The number of cycles required to cause liquefaction at different stress ratios was found to be typical for such materials, and in all tests, post liquefaction shearing of the samples indicated a continuous gain in strength, or strain hardening, due to the strongly dilative nature of the material.

Using the results of this testing, analyses demonstrated that the tailings facility will be stable under most conditions with a small probability of an earthquake induced strength reduction during the operating life of the mine. The analyses were based on the assumptions of a normally consolidated saturated tailings material. The overconsolidation induced in the tailings by the deposition technique will result in an additional factor of safety against movement. The consequences of any strength reduction are not, however, severe, due to the strain hardening nature of the material which eliminates the possibility of mud-flow type of failure. The maximum probable displacements under the 200-year and 475-year design earthquakes have been estimated at 0.86 and 6.0 m, respectively. The provision of a sufficiently wide and erosion resistant starter embankment to the tailings faciltiy will ensure that potential adverse consequences of such displacements are negligible.

Waste rock from the H-W Mine has been examined and has been confirmed as an excess acid producer and is capable of sustaining acid generation. It is planned to dispose of this rock on or adjacent to the present Lynx open pit waste rock dumps where any leachate can be managed by the surface and groundwater collection system. Following the first years of expanded production, there will be a projected shortfall in backfill for mining requirements and, it is at this time proposed to utilize the Lynx open pit high pyrite waste rock. This option is to be examined in greater detail when backfill requirements are more fully delineated.

On commissioning of the new mill in late 1984, a large disposal area will be required, which will be provided by diverting a section of Myra Creek and extending the storage area towards the south. The 200-year floodplain limits and Maximum Probable Flood Limits (MPF) for both the natural and proposed diversion channels have been established. The design of all facilities adjacent to the natural channel and the proposed diversion, including the tailings area starter embankment, the Myra integrated water management system pond berms and stream crossings, incorporate these flood level considerations. The conceptual design for the diversion has also taken into account the need to replace lost habitat features such as pool, riffle and glide sequences, habitat cover and shoreline vegetation, spawning, rearing and food production areas.


TRUE
N
CREEK
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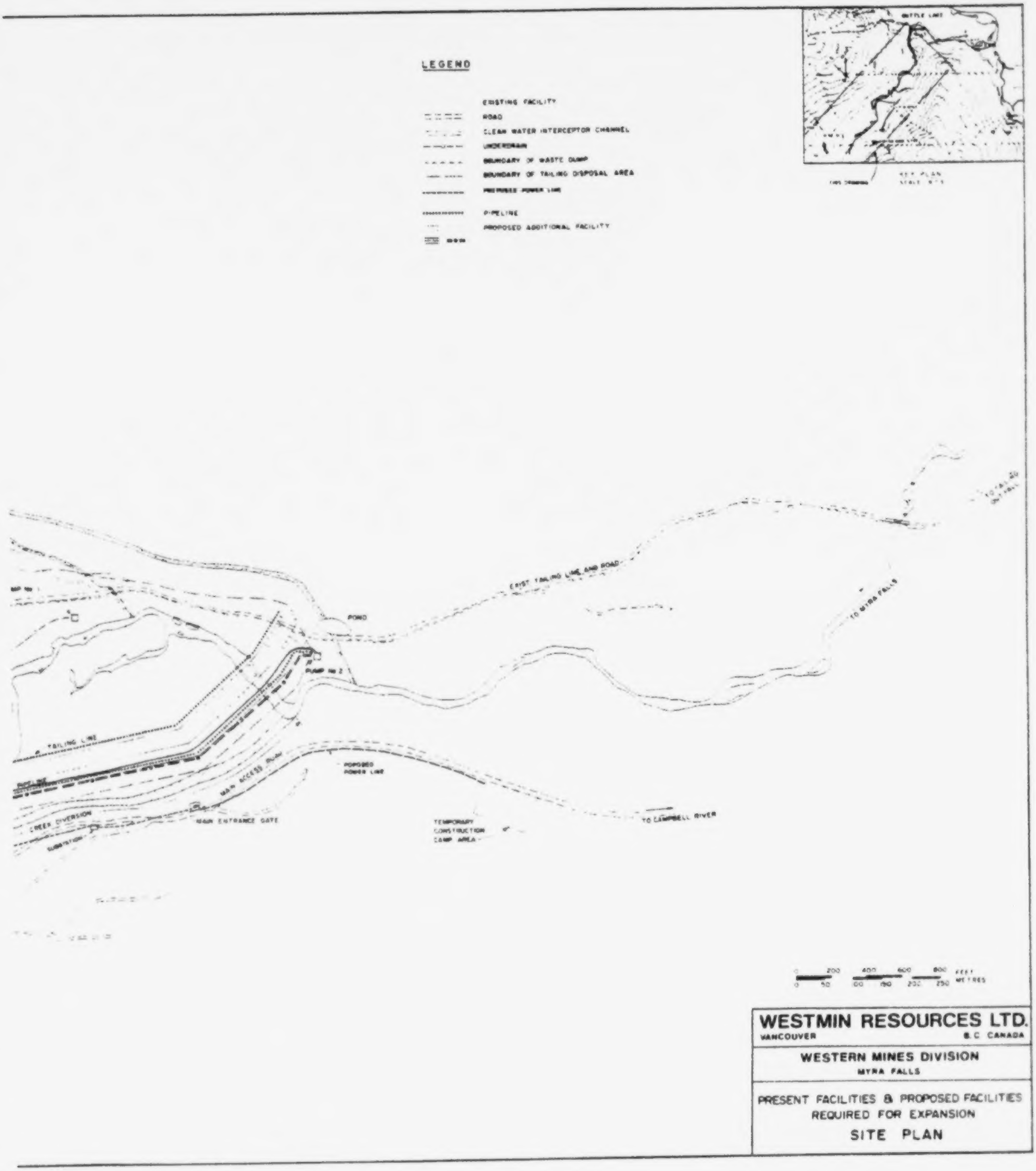
WASTE DUMP No.2
WASTE DUMP No.1
LYNX HEAD TANK
FRESH AND RECLAIMED WATER TANKS
CRUSHER BLDG
CONV. No.2
CONV. No.3
SUBSTATION
DIESEL POWER PLANT
NEW MILL
EXISTING MILL
THICKENER
ASSAY LAB
O.C. PLANT
MAIN SUBSTATION
TAILING LINE
CONVEYOR No.1
SETTLING PONDS
1A
1
2
3
4
SEWAGE TREATMENT PLANT
TO HYDRO POWER PLANT
DYKE
SURGE BASIN
PUMP No.3
SLUDGE DISPOSAL AREAS
EXIST. TAILING
PLAN No.1
TAILING DISPOSAL AREA
PIPE BRIDGE
MAIN ACCESS ROAD
MYRA CREEK
PROPOSED POWER LINE
INTEGRATED WATER TREATMENT PONDS
MIXING TANK
SUBSTATION
MYRA 0 LEVEL
MYRA

Figure 1. Site plan of Westmin Resources Ltd., Myra Falls operations.


LEGEND
EXISTING FACILITY
ROAD
CLEAN WATER INTERCEPTOR CHANNEL
UNDERDRAIN
BOUNDARY OF WASTE DUMP
BOUNDARY OF TAILING DISPOSAL AREA
PROPOSED POWER LINE
PIPELINE
PROPOSED ADDITIONAL FACILITY
KEY PLAN
EXIST. TAILING LINE AND ROAD
POND
PUMP No 2
TO MYRA FALLS
TAILING LINE
PIPELINE
CREEK DIVERSION
SUBSTATION
MAIN ENTRANCE GATE
MAIN ACCESS ROAD
PROPOSED POWER LINE
TEMPORARY CONSTRUCTION CAMP AREA
TO CAMPBELL RIVER
0 200 400 600 800 FEET
0 50 100 150 200 250 METRES

WESTMIN RESOURCES LTD.
VANCOUVER B.C. CANADA

WESTERN MINES DIVISION
MYRA FALLS

PRESENT FACILITIES & PROPOSED FACILITIES
REQUIRED FOR EXPANSION
SITE PLAN

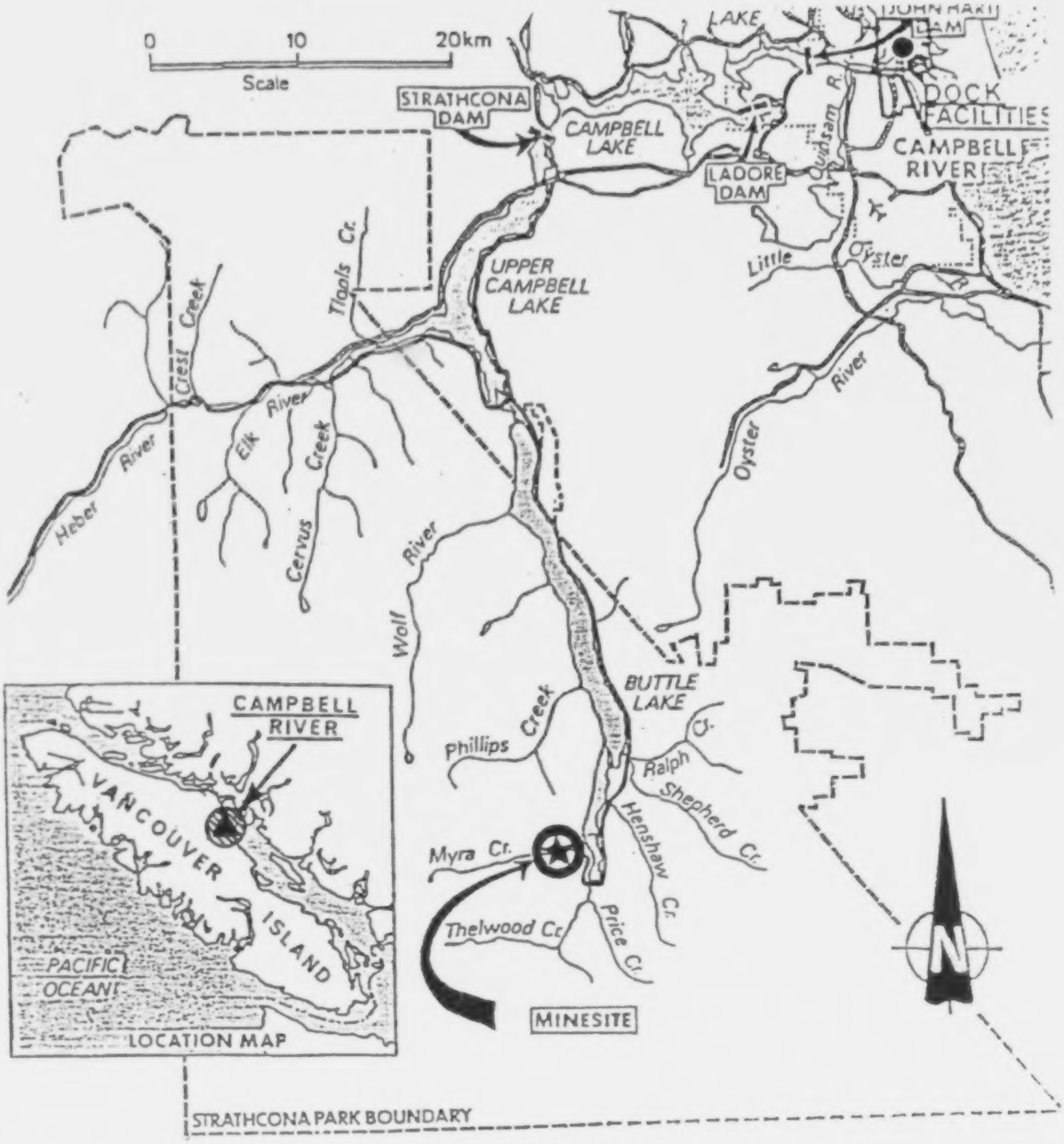

0
10
20km
Scale
STRATHCONA DAM
CAMPBELL LAKE
JOHN HART DAM
DOCK FACILITIES
CAMPBELL RIVER
LADORE DAM
Quinsam R.
Little Oyster R.
UPPER CAMPBELL LAKE
Tlools Cr.
Crest Creek
Heber River
Elk River
Cervus Creek
Wolf River
Oyster River
BUTTLE LAKE
Phillips Creek
Ralph Cr.
Shepherd Cr.
Henshaw Cr.
Myra Cr.
Thelwood Cr.
Price Cr.
MINESITE
N
CAMPBELL RIVER
VANCOUVER ISLAND
PACIFIC OCEAN
LOCATION MAP
STRATHCONA PARK BOUNDARY

Figure 2. Location map of Westmin Resources Ltd., Myra Falls operations.

A LABORATORY STUDY OF THE BIOACCUMULATION OF METALS BY BIVALVES EXPOSED TO ALICE ARM SEDIMENT CONTAMINATED WITH AMAX/KITSAULT MINE TAILINGS

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The bioaccumulation of cadmium (Cd), copper (Cu), lead (Pb), molybdenum (Mo) and zinc (Zn) by two species of marine bivalves (filter-feeding mussels, *Mytilus edulis*; and detrital-feeding *Yoldia limatula*) exposed to Alice Arm sediment contaminated with Amax/Kitsault mine tailings or to an uncontaminated reference sediment, was assessed in the laboratory. Both bivalve species were analysed for whole-body tissue metal concentrations after exposure periods of 2, 4, 7, 14, 31, 60 or 90 days. Small and large-sized specimens of each species were analysed separately in order to identify the effect of size difference on whole-body tissue metal levels.

Elevated tissue metal concentrations in mussels were attributed to dissolved metals released from Alice Arm sediment; whereas those in the benthic clam species examined were thought to reflect direct uptake of metals from the sediment ingested as well as that from dissolved metals. For each of the five metals examined, metal concentrations and rates of uptake from Alice Arm sediment into whole-body tissue differed due to species. Replicate values for identical treatments were always similar. For both bivalve species, the degree of bioaccumulation of metals showed the same relationship: Pb>Mo>Cd>Zn>Cu.

Metal concentrations were generally higher (up to three times) in smaller (younger) specimens. No overt toxic effects toward either bivalve species associated with metal uptake were apparent in this study.

Tissue metal losses during depuration in uncontaminated seawater of *Yoldia* sp. previously exposed to Alice Arm sediment for 60 days showed half-lives for elimination of accumulated tissue metal of 2 (Pb), 6 (Mo), 7 (Zn) or >7 (Cd, Cu) days.

The findings from this study are discussed in terms of environmental and human health concerns associated with the discharge of mine tailings to the aquatic environment. Recommendations for future laboratory and *in situ* bioaccumulation studies will be made.

UNE ÉTUDE EN LABORATOIRE SUR L'ACCUMULATION, DANS LES TISSUS VIVANTS, DE MÉTAUX DANS DES BIVALVES EXPOSÉS AUX SÉDIMENTS D'ALICE ARM CONTAMINÉS PAR LES DÉCHÊTS MINIERS D'AMAX/KITSAULT

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On a estimé en laboratoire l'accumulation du cadmium (Cd), cuivre (Cu), plomb (Pb), molybdène (Mo) et zinc (Zn) dans les tissus de deux espèces de bivalves marins (Mytilus edulis - filtreur, et Yoldia limatula - détritivore) exposés soit aux sédiments d'Alice Arm, lesquels sont contaminés par les déchets miniers d'Amax/Kitsault, soit à des sédiments de référence non contaminés. Les concentrations de métaux dans les tissus des bivalves furent déterminées après des périodes d'exposition de 2, 4, 7, 14, 31, 60 ou 90 jours. Des spécimens (petits et gros) de chaque espèce furent analysés séparément en vue d'identifier l'effet de différentes grosseurs sur les niveaux de métaux dans les tissus.

Les fortes concentrations de métaux dans les tissus des moules furent attribuées à la libération de métaux dissous par les sédiments d'Alice Arm, tandis que celles dans les espèces de clams benthiques examinées furent considérées comme étant le reflet autant de l'absorption directe de métaux à partir de sédiments ingérés que de l'absorption de métaux dissous. Les concentrations de métaux et les taux d'absorption des sédiments d'Alice Arm dans les tissus différaient selon l'espèce, et ceci pour chacun des cinq métaux examinés. Les valeurs de traitements identiques répétés furent toujours similaires. Le degré de bioaccumulation des métaux suivait la même relation pour les deux espèces de bivalves: Pb>Mo>Cd>Zn>Cu.

Les concentrations de métaux étaient généralement plus hautes (jusqu'à trois fois) dans les petits (jeunes) spécimens. Cette étude n'a révélé aucun effet toxique apparent associé à l'absorption de métaux par les deux espèces de bivalves en question.

Pour les Yoldia sp. ayant été exposées aux sédiments d'Alice Arm pour 60 jours, l'élimination de métaux accumulés dans les tissus durant la dépuration en eau de mer non contaminée avait des temps de demi-vie de 2 (Pb), 6 (Mo), 7 (Zn) ou >7 jours (Cd, Cu).

Les résultats de cette étude seront discutés en termes d'intérêts environnementaux et de santé humaine associés à la décharge des déchets miniers dans l'environnement aquatique. Des recommandations pour de futures études en laboratoire et pour des études sur la bioaccumulation in situ seront proposées.

EXTENDED ABSTRACT

The bioaccumulation of cadmium (Cd), copper (Cu), lead (Pb), molybdenum (Mo) and zinc (Zn) by two species of marine bivalves (filter-feeding mussels, *Mytilus edulis*; and detrital-feeding *Yoldia limatula*) exposed to Alice Arm sediment contaminated with Amax/Kitsault mine tailings, or to a reference (Satellite Channel) sediment, was assesssed in a laboratory study. Both bivalve species were sampled for whole-body tissue metal analyses after exposure periods of 2, 4, 7, 14, 31, 60, or 90 days. Seawater temperature, pH, dissolved oxygen and salinity within test aquaria were similar to those within Alice Arm.

The extent to which metals accumulated in whole-body tissues of each bivalve species due to their exposure to Alice Arm sediment (relative to tissue metal values for corresponding groups of bivalves exposed to the reference sediment) was as follows: Pb>Mo>Cd>Zn>Cu. No bioaccumulation of copper was evident for mussels held in seawater overlying this sediment source. The elevated tissue metal concentrations in mussels were attributed to dissolved metals released from Alice Arm sediment; whereas those in *Yoldia* sp. were thought to reflect direct uptake from the sediment ingested as well as that from dissolved metals.

Tissue zinc concentrations for *Mytilus* sp. held in seawater overlying Alice Arm sediment increased progressively throughout the test period. Cadmium and lead concentrations appeared to approach equilibrium after 90 days of exposure, whereas tissue Mo values for this species reached a state of equilibrium within 7 days. Tissue Cd and Cu values for *Yoldia* sp. exposed to Alice Arm sediment also remained elevated after rapid (within 7 days) increases; whereas tissue Pb, Mo, Zn concentrations for this species declined after an initial increase in the first 14-31 days of the test.

For each species of bivalve, the degree of metal bioaccumulation was inversely related to the size of the individuals examined. Small mussels suspended above Alice Arm sediment accumulated appreciably (up to 3 times) higher concentrations of each of the five metals relative to corresponding values for large specimens. Small *Yoldia* sp. also showed a greater uptake of Cd, Pb, and Zn relative to large-sized specimens, whereas tissue Mo concentrations for this species were unaffected by size. Copper concentrations in small clams were consistently lower than corresponding values for larger specimens.

The effect of differing depuration periods on tissue metal concentrations was determined for *Yoldia* sp. held in uncontaminated seawater for 2-7 days following a 60-day exposure to Alice Arm sediment. Residual quantities of sediment retained in the guts of clams depurated for 2 days contributed to 3% or less of their measured whole-body concentrations of Cd, Cu, Pb, or Zn, with a lesser sediment contribution when the depuration period was extended. Greater contributions to tissue Mo values were caused by residual gut sediment. Differing rates for elimination of the accumulated metals were evident during this 7-day depuration period. Biological half-lives calculated (after adjustment for gut sediment) were 2 (Pb), 6 (Mo), 7 (Zn), or >7 (Cd, Cu) days.

The survival and condition factors for each species of bivalve were not affected by metals accumulated from the Alice Arm sediment.

Our laboratory data indicate that cadmium and lead can accumulate to a high degree in whole-body tissue of the edible blue mussel, due to its prolonged exposure to seawater overlying Alice Arm sediment. Based on this finding and on recommended limitations for dietary intake of these metals, additional monitoring of cadmium and lead concentrations in mussels and other edible bivalve species within Alice Arm and adjacent waters is advised. No human health concerns were evident with respect to the maximum concentrations of copper, molybdenum or zinc found in exposed mussels.

Test results were discussed in relation to the influence of environmental variables on metal bioavailability and with respect to whole-body tissue concentrations of these metals found in samples of _Mytilus edulis_ and _Yoldia_ sp. collected from Alice Arm.

A REVIEW OF TOXICITY AND TISSUE RESIDUES OF LEAD, ZINC AND CADMIUM FOR MARINE MOLLUSCS AND CRUSTACEANS

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A search of the primary literature turned up about 1000 relevant papers. Many of them seemed repetitive or lacking in hypotheses, and it did not appear urgent to grind out more such numbers. There was generally poor documentation of the environmental levels of metals that were associated with the reported tissue levels. Variation in toxicity or residue often spanned two orders of magnitude, so geometric averages have been used in an attempt to generalize from these data.

The acute LC_{50} of lead for shellfish may be about 10 mg/L and sublethal effects may occur at about 0.26 mg/L, compared to a solubility of less than 1 mg/L in seawater. Lethal concentrations of zinc averaged 1-10 mg/L for various groups of organisms, and sublethal values were 0.2 and 0.4 mg/L for molluscs and crustaceans, respectively. Measurement of cadmium toxicity was popular; lethal levels averaged 0.5-6 mg/L and sublethal ones 0.05-0.23 mg/L. In general, statistical differences could not be demonstrated in toxicity to different groups (oysters, clams, snails, etc.). Any small differences would be obscured by variation in testing, for example an average 8-fold range in LC_{50}s for the same species from different laboratories. Some apparent differences may have been artifacts of test-duration, for example a 4-day test would represent a sub-acute exposure for copepods but an acute one for crabs.

All possible results, from more-than-additive to antagonistic, were obtained for joint toxicity of metals, or metals with other toxicants. The usual situation in polluted marine locations is probably the simultaneous presence of several toxicants, and this topic deserves much more evaluation to improve our predictive ability.

There were 1100 values for whole-body residues of metals, three-quarters of them for molluscs. Organisms were lumped into 15 convenient taxonomic groups of molluscs and 5 of crustaceans, since preliminary analyses had indicated than an oyster was an oyster, etc., worldwide without regard to species.

Most measurements of lead in clean-water shellfish fell in the range 1-50 mg/kg dry weight. Most molluscs including oysters, mussels and clams were similar in lead content, although whelks were statistically lower than several other groups. Crustaceans showed no differences between groups and were similar in residues to the molluscs. Average lead content usually doubled, at least, in organisms from so-called "polluted" locations. Differences between clean and polluted molluscs were usually significant despite large overlaps. Mussels appeared to be good indicators of pollution, judging by their lead content.

Zinc residues of 50-500 mg/kg were found for most clean-water organisms, however oysters averaged 2,000 mg/kg, significantly higher than all other molluscs, and barnacles 1,000 mg/kg, higher than other crustaceans. Both these groups showed further significant increases in polluted locations, with extreme values of 5% zinc in some Australian oysters and 11% in some Welsh barnacles!

Clean-water cadmium residues could be generalized as ranging from 0.5 to 50 mg/kg in molluscs and 0.2-10 mg/kg in crustaceans. Drills were significantly higher than most other molluscs. Scallops were puzzlingly high but this was not shown to be significant. There were no statistical differences among the crustaceans. There was an overall significant increase in cadmium residues for polluted molluscs, but not for crustaceans. Oysters were again the outstanding indicators with an average value of 18 mg/kg in polluted locations, but clams also appeared to be fairly good indicators.

Overall, metal residues varied as much within groups as between most groups; an analysis based on trophic relationships might be more revealing, if it were possible. The mussel watch program should be giving a good indication of metal pollution. Oysters are excellent indicators for zinc and cadmium, and barnacles for zinc and perhaps other metals. Tellin clams also appear to be promising indicators. For other shellfish, it seems difficult to document changes in residues with "pollution", and snails in particular are not good indicators.

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REVUE DE LA TOXICITÉ ET DES RÉSIDUS DE PLOMB, ZINC ET CADMIUM AFFECTANT LES MOLLUSQUES ET CRUSTACÉS MARINS

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Une revue de la littérature primaire révéla jusqu'à environ 1000 articles relevants. Plusieurs de ceux-ci apparaissaient répétitifs ou déficients en hypothèses, et il ne sembla pas nécessaire de générer encore plus de données similaires. La documentation sur les niveaux de métaux trouvés dans le milieu et étant associés avec les niveaux reportés pour les tissus était généralement pauvre. Etant donné que la variation dans la toxicité ou dans les résidus chevauchait souvent deux ordres de magnitude, on a utilisé des moyennes géométriques pour essayer d'en arriver à une généralisation à partir de ces données.

Les CL_{50} aigus du plomb pour les coquillages sont d'à peu près 10 mg/L et les effets sub-létaux peuvent survenir à environ 0.26 mg/L, comparé à une solubilité de moins de 1 mg/L en eau salée. Les concentrations létales de zinc avaient une moyenne de 1 à 10 mg/L pour plusieurs groupes d'organismes, et les valeurs sub-létales étaient de 0.2 et 0.4 mg/L pour les mollusques et les crustacés respectivement. Les mesures de toxicité de cadmium étaient populaires: les niveaux létaux avaient une moyenne de 0.5 à 6 mg/L et les niveaux sub-létaux variaient entre 0.05 et 0.23 mg/L. En général, aucune différence statistique en toxicité ne peut être démontrée pour différents groupes (huîtres, moules, escargots, etc.). Une petite différence serait masquée par la variation dans la méthode d'analyse: par exemple, les CL_{50} pour la même espèce varient par un facteur de 8 entre différents laboratoires. Certaines différences peuvent être des artefacts de durée des tests: par exemple, une analyse de 4 jours pourrait représenter une exposition non dommageable pour des copépodes mais très sévère pour des crabes.

Toute la gamme des résultats possibles, en passant des plus additifs jusqu'aux antagonistes, fut obtenue pour la toxicité combinée des métaux, ou des métaux avec d'autres substances toxiques. La situation actuelle existant dans les locations marines polluées en est probablement une où plusieurs substances toxiques sont présentes simultanément, et ce sujet mérite qu'on s'y attarde beaucoup plus afin d'améliorer notre pouvoir de prédiction.

Il y avait 1100 valeurs pour les résidus de métaux dans tout l'organisme, dont trois quarts de celles-ci étaient pour les mollusques seulement. Les organismes furent groupés en 15 groupes taxonomiques pour les mollusques et en 5 groupes pour les crustacés, étant donné que des analyses préliminaires avaient indiqué qu'une huître demeure une huître, etc., et ceci à travers le monde sans égard pour l'espèce.

La plupart des mesures de plomb dans les coquillages d'eau non polluée variaient entre 1 et 50 mg/kg de poids sec. La plupart des mollusques, incluant les huîtres, les moules et les clams, avaient un contenu en plomb similaire bien que les buccins avaient un contenu statistiquement plus bas que celui de plusieurs autres groupes. Les crustacés ne montraient aucune différence entre les groupes et étaient similaires aux mollusques au point de vue résidus. Le contenu moyen en plomb des organismes provenant de locations soi-disant 'polluées' doublait, d'habitude. Les différences entre mollusques d'eaux claires et polluées étaient habituellement significatives, malgré d'importants chevauchements. A en juger par leur contenu en plomb, les moules semblaient être de bons indicateurs de pollution.

On trouva des résidus en zinc de 50 à 500 mg/kg pour la plupart des organismes d'eau claire, bien que les huîtres aient une moyenne de 2000 mg/kg, ce qui est significativement plus haut que pour les autres mollusques, et les anatifes (balanes) 1000 mg/kg, ce qui est aussi plus haut que les valeurs trouvées pour les autres crustacés. Ces deux derniers groupes montrèrent, de plus, des augmentations significatives dans les locations polluées, avec des valeurs extrêmes de 5% dans certaines huîtres australiennes et de 11% dans certains anatifes du pays de Galle.

On peut généraliser les résultats pertinents aux résidus de cadmium en eau claire en disant qu'ils varient de 0.5 à 50 mg/kg pour les mollusques et de 0.2 à 10 pour les crustacés. Les mollusques foreurs avaient des résultats significativement plus hauts que ceux de la plupart des autres mollusques. Les pétoncles étaient anormalement hautes mais ce résultat ne s'avéra pas significatif. Il n'y avait aucune différence statistique parmi les crustacés. Il y avait une augmentation générale dans les résidus de cadmium parmi les mollusques de milieux pollués, mais non pas pour les crustacés. Les huîtres étaient encore une fois des indicateurs frappants avec une valeur moyenne de 18 mg/kg dans les locations polluées, mais les clams aussi semblaient être de bons indicateurs.

En général, les résidus de métaux variaient autant à l'intérieur d'un groupe qu'entre la plupart des groupes; une analyse basée sur les relations trophiques pourrait s'avérer plus révélatrice, en admettant qu'elle soit possible. Le programme de surveillance des moules devrait fournir une bonne indication de la pollution par les métaux. Les huîtres sont d'excellents indicateurs de zinc et de cadmium, et les anatifes le sont pour le zinc et peut-être pour d'autres métaux. Les clams tellines promettent aussi comme indicateurs. Il semble difficile de documenter les changements dans les résidus avec "pollution" pour les autres coquillages, et les escargots en particulier, ne sont pas de bons indicateurs.

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ACUTE TOXICITY OF IRON CYANIDE SPECIES TO RAINBOW TROUT (SALMO GAIRDNERI) AND TO DAPHNIA MAGNA UNDER EXPOSURE TO VARIOUS LIGHT INTENSITIES

Clark, M.J.R., H. Hanssen, G. van Aggelen and S. Horvath. B.C. Ministry of Environment, Waste Management Br., Victoria, B.C.

A major environmental concern regarding cyanides is the photochemical conversion of relatively non-toxic metallocyanides such as ferrocyanide and ferricyanide ions into more toxic forms such as hydrogen cyanide (HCN) and cyanates (CNO^-). The degree of environmental impact of cyanides is a concern in British Columbia because of their widespread presence in effluents from the base metal mining activities in the province. A laboratory system has been developed to determine toxicity levels of iron cyanide complexes in mining and industrial effluents to rainbow trout (*Salmo gairdneri*) and to *Daphnia magna* under dark, laboratory light and artificial light source with a spectral emission similar to sunlight. Toxicity levels and chemical speciation (by ion chromatography) are determined for various exposures to light intensity and temperature conditions. There is a great difference in results between bioassays conducted under various conditions, and much of the published bioassay data for metallocyanide complexes must now be viewed as too conservative for appropriate protection of the environment.

TOXICITÉ AIGUË DE CYANURES FERREUX ENVERS LA TRUITE ARC-EN-CIEL (SALMO GAIRDNERI) ET DAPHNIA MAGNA SOUS EXPOSITION À DIVERSES INTENSITÉS LUMINEUSES

Clark, M.J.R., H. Hanssen, G. van Aggelen et S. Horvath. B.C. Ministry of Environment, Waste Management Br., Victoria, B.C.

Une préoccupation environnementale majeure concernant les cyanures est la conversion photochimique de métallocyanures relativement non toxiques tels les ions ferrocyanure et ferricyanure en des formes plus toxiques telles le cyanure d'hydrogène (HCN) et les cyanates (CNO^-). Le degré d'impact environnemental des cyanures est une préoccupation importante en Colombie Britannique, parce que leur présence est répandue due aux écoulements de métaux de base provenant des activités minières dans la province. Un système en laboratoire a été développé pour déterminer les niveaux toxiques, pour la truite arc-en-ciel (*Salmo gairdneri*) et *Daphnia magna*, des complexes de cyanure de fer dans les écoulements miniers et industriels sous lumière très tamisée en laboratoire et sous une source artificielle ayant une émission spectrale similaire à la lumière du soleil. Les niveaux de toxicité et la spéciation chimique (par chromatographie ionique) sont déterminés sous différentes expositions lumineuses et sous différentes températures. Il existe de grandes différences dans les résultats de bioassais conduits sous différentes conditions, et par conséquent la plupart des données de bioassais publiées sur les complexes de métallocyanures doivent maintenant être considérées trop conservatrices pour mener à une protection adéquate de l'environnement.

ACUTE LETHAL MARINE BIOASSAY STUDIES FOR THE U.S. BORAX QUARTZ HILL PROJECT

Mitchell, D.G.[1], J.D. Morgan[1], J.L. Cronin[2], D.A. Cobb[3], G.A. Vigers[1] and P.M. Chapman[1]

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The acute lethality of mine tailings produced by pilot plant operations for the U.S. Borax Quartz Hill Molybdenum Mine was determined. Species tested represented pelagic, intertidal and benthic environments: juvenile coho salmon (Oncorhynchus kisutch), mussel larvae (Mytilus edulis), amphipod (Rhepoxynius abronius) and zooplankter (Euphausia pacifica). The acute toxicity of the mine tailings was low for all four species tested with acute effects observed between 61,000 and 277,000 mg/L tailings solids (range of 95% confidence limits for LC_{50} and EC_{50} values), representing tailings slurry to seawater ratios of 1:1 to 1:7. General trends within this range of concentrations indicated the following relative species sensitivities:

Least Sensitive		Most Sensitive

coho < amphipods & mussel larvae < euphausiids

Coincident tests with a reference toxicant (sodium pentachlorophenate) supported the relative species sensitivities, with the exception of the amphipod tests, for which sediment acted as a modifying factor. Chemical analyses of mine tailings and test solutions implicated manganese, molybdenum and process reagents as possible constituents responsible for the observed toxicity.

ÉTUDES BIOLOGIQUES MARINES SUR LA MORTALITÉ AIGUË POUR LE PROJET U.S. BORAX QUARTZ HILL

Mitchell, D.G.[1], J.D. Morgan[1], J.L. Cronin[2], D.A. Cobb[3], G.A. Vigers[1], et P.M. Chapman[1]

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On a déterminé la mortalité aiguë causée par les déchets miniers produits par les opérations du projet pilote de U.S. Borax Quartz Hill Molybdenum Mine. Les espèces analysées étaient représentatives des environnements pélagiques, intertidaux et benthiques: saumons juvéniles coho (Oncorhynchus kisutch), larves de moules (Mytilus edulis), amphipodes (Rhepoxynius abronius) et du zooplancton (Euphausia pacifica). La toxicité aiguë des déchets miniers était basse pour les quatre espèces analysées, les effets aigus étant observés entre 61,000 et 277,000 mg/L de solides de déchets (intervalle de limite de confiance de 95% pour les valeurs de CL_{50} et CI_{50}), ce qui représente un rapport déchet: eau de mer de 1:1 à 1:7. Les sensibilités relatives des espèces soumises à ces variations de concentration sont, de moins sensible à plus sensible:

coho < amphipodes et larves de moules < euphausides

Les analyses coïncidentes faites avec un produit toxique de référence (pentachlorophénate de sodium) supportent les sensibilités spécifiques relatives, à l'exception des tests menés sur les amphipodes, pour lesquels le sédiment agissait comme un facteur modificateur. Les analyses chimiques des déchets miniers et des solutions essai pointent vers le manganèse, le molybdène et les réactifs du procédé comme éléments constitutifs potentiellement responsables de la toxicité observée.

PHYTOPLANKTON, ZOOPLANKTON AND DISSOLVED METALS IN THE LAKES OF THE CAMPBELL RIVER WATERSHED, VANCOUVER ISLAND

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Four lakes on Vancouver Island were investigated to determine the effects of increased concentrations of zinc, copper, lead and cadmium from a mining operation on the aquatic biota of the lakes. A distinct gradient of metals exists which is highest near the mine at the upper end of the watershed. The concentrations decrease with distance downstream. Phytoplankton primary production at the two stations nearest the mine was significantly depressed in comparison to more downstream stations. Zooplankton standing crop also displayed a similar pattern with lower biomass closer to the mine. Bioassays with Daphnia showed toxicity highest near the mine. Historical data show changes in species composition of both the phytoplankton and zooplankton communities. Concentration of metals in the tissues of zooplankton, sticklebacks and trout were also measured. Zooplankton showed the highest concentration factors and both zinc and copper tissue concentrations are significantly correlated with metal concentrations in the water. Metal in sticklebacks also declined with distance from the mine. Metals in trout liver appeared to be a better indicator of metal contamination than concentration of metals in muscle tissues. Overall evidence indicates that more severe effects have occurred in phytoplankton and zooplankton than the fish community and the lower trophic levels appear to be more sensitive indicators of the effects of metals.

PHYTOPLANCTON, ZOOPLANCTON ET MÉTAUX DISSOUS DANS LES LACS DU BASSIN DE LA RIVIÈRE CAMPBELL, SUR L'ÎLE DE VANCOUVER

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On a étudié quatre lacs sur l'île de Vancouver dans le but de déterminer l'effet de l'augmentation des concentrations de zinc, cuivre, plomb et cadmium provenant d'une opération minière, sur le milieu aquatique de ces lacs. Un clair gradient des métaux existe, avec son point le plus haut près de la mine, à la partie supérieure de bassin. Les concentrations diminuent avec la distance en aval. La production primaire de phytoplancton aux deux stations les plus près de la mine était significativement plus en déclin, comparée aux stations plus en aval. Le zooplancton présent avait une distribution similaire avec une biomasse plus basse près de la mine. Des bioassais avec *Daphnia* révélèrent une haute toxicité près de la mine. Les données historiques montrent des changements dans la composition des espèces des communautés de phytoplancton et de zooplancton. On mesura aussi la concentration des métaux dans les tissus de zooplancton, d'épinoches et de truites. Le zooplancton avait les plus hautes concentrations et une corrélation significative fut établie entre les concentrations de zinc et de cuivre dans les tissus et, les concentrations de métaux dans l'eau. Les métaux dans les épinoches déclinaient aussi avec la distance de la mine. Les métaux dans le foie des truites apparaissent être un meilleur indicateur de contamination par le métal que la concentration de métaux dans les tissus musculaires. En général, des effets plus profonds ont lieu dans le phytoplancton et dans le zooplancton que dans la communauté des poissons et, les niveaux trophiques les plus bas apparaissent être des indicateurs plus sensibles des effets des métaux.

METALLOTHIONEIN INDUCTION, GROWTH AND SURVIVAL OF RAINBOW TROUT EXPOSED TO MIXED HEAVY METAL CONTAMINATION

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Rainbow trout were exposed to mixed heavy metal contamination (zinc, copper, cadmium) in situ and in laboratory exposures for 4 weeks and in longer exposures (16 weeks) from hatch. Metallothionein concentration in the livers of the rainbow trout showed a good correlation with the metal concentration in the water. Earlier measurements of hepatic metallothionein in wild rainbow trout had also shown a good correlation with metal concentrations in a lake which is contaminated with heavy metal due to acid mine drainage. Laboratory exposures showed that the rainbow trout acclimated to the mixture of metals. No increase in tolerance could be demonstrated after in situ exposure.

Individual 96 h LC_{50}'s as compared to mixtures of zinc, copper and cadmium indicate that the mixture is moderately antagonistic or that the LC_{50} of the mixture in the ratio 400:200:1 (Zn:Cu:Cd) is comparable to the LC_{50} for zinc alone.

Rainbow trout were more sensitive at the swim-up stage than at hatch and had acclimated to metals within 4 weeks of hatch. Growth and survival were not inhibited by a concentration of 120 µg Zn, 6 µg Cu and 0.2 µg Cd/L in soft water (25 mg/L as $CaCO_3$) at 12°C during a 16 week exposure from hatch.

Comparative bioassays have shown that different stocks of rainbow trout and steelhead may vary markedly in susceptibility to metals. Safe concentration applied to a particular contaminated site should be based on the stocks that occur in that region.

INDUCTION DE MÉTALLOTHIONÉINE, CROISSANCE ET SURVIE DE TRUITES ARC-EN-CIEL EXPOSÉES À LA CONTAMINATION DE MÉLANGES DE MÉTAUX LOURDS

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Des truites arc-en-ciel furent exposées *in situ* et en laboratoire à un mélange de métaux lourds (zinc, cuivre, cadmium) pour des périodes de 4 et 16 semaines après éclosion. Une bonne correlation fut établie entre la concentration de métallothionéine dans le foie des truites arc-en-ciel et la concentration de métaux dans l'eau. Des mesures de métallothionéine hépatique prises plus tôt à partir de truites arc-en-ciel sauvages avaient aussi montré une bonne corrélation avec les concentrations de métaux présents dans un lac contaminé par des métaux lourds provenant de résidus miniers acides. Des expositions en laboratoire montrèrent que la truite arc-en-ciel s'acclimate au mélange des métaux. On ne put démontrer aucune augmentation dans le niveau de tolérance après l'exposition *in situ*.

Des CL_{50} individuels de 96 h, comparés à des mélanges de zinc, cuivre et cadmium, indiquent que le mélange est modérément antagoniste ou que le CL_{50} du mélange dans le rapport 400:200:1 (Zn:Cu:Cd) est comparable au CL_{50} pour le zinc seul.

Les truites arc-en-ciel étaient plus sensibles durant l'étape de montée qu'à l'éclosion et s'acclimataient aux métaux en dedans de 4 semaines après éclosion. La croissance et la survie n'étaient pas inhibées par une concentration de 120 µg de Zn, 6 µg de Cu et de 0.2 µg de Cd/L dans de l'eau douce (25 mg/L en $CaCO_3$) à 12°C durant 16 semaines d'exposition commençant à l'éclosion.

Des bioassais comparatifs ont montré que différentes populations de truites arc-en-ciel et de truites arc-en-ciel anadromes ('steelhead') peuvent varier de façon marquée dans leur susceptibilité aux métaux. La concentration infligée à un site contaminé particulier devrait être basée sur le type de population qui existe dans cette région.

ACIDIFICATION

PAPERS AND ABSTRACTS

CHANGES IN THE ALUMINUM CONTENT OF TISSUES OF CRAYFISH HELD IN THE LABORATORY AND IN EXPERIMENTAL FIELD ENCLOSURES

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Studies of bioaccumulation and elimination of aluminum from the tissues of the crayfish, Orconectes virilis, were conducted in mid-summer at the Experimental Lakes Area (ELA), northwestern Ontario.

Crayfish from an unmodified ELA Lake 239, (average [Al] in water, 36 µg/L) were placed in cages in 1 m diameter tubes in Lake 302 (average [Al], 8 µg/L). These tubes were spiked with Al (40 µg/L) or Cd (1 or 3 µg/L) or kept as controls without metal addition. Half of the tubes in each metal treatment were acidified to pH 5.3; the others remained at pH 6.7. None of the crayfish in tubes accumulated Al in the tissues. Crayfish held in tubes without Al addition had lower [Al] in hepatopancreas and abdominal muscle after 25 to 27 d exposure than did freshly-captured crayfish. Crayfish in the 40 µg/L Al, pH 5.3 tube retained background [Al] in hepatopancreas but not in muscle. Crayfish in the 40 µg/L Al, pH 6.7 tube retained [Al] in the muscle but not in the hepatopancreas. Considering all metal treatments, pH did not have a clear effect on [Al] in tissues. The addition of Cd in some tubes did not affect [Al] in tissues of crayfish.

In a laboratory experiment, [Al] was measured in the tissues of three groups of crayfish obtained from Lake 239. The first group were dissected within a few hours after capture for background [Al] in six tissues. The second group was dissected following exposure to 500 µg/L in the laboratory for 14 days. None of the tissues showed an increase in [Al]. The third group were exposed to 500 µg/L Al for 14 d then were held in Lake 239 water for an additional 16 days. Crayfish were not fed during the experiment. Over the 30 d exposure only carapace and gills retained background [Al]. All other tissues had lower [Al] at the end of 30 d. The total [Al] in the water does not appear to control tissue [Al]. The results of these experiments lead to the hypothesis, presently being tested, that the gut is the most important site of Al uptake by the crayfish, either from the food iself or from inorganic matter incidentally ingested.

CHANGEMENTS DANS LE CONTENU EN ALUMINIUM DE TISSUS PROVENANT D'ÉCREVISSES GARDÉES EN LABORATOIRE ET DANS DES ENCEINTES EXPÉRIMENTALES SUR LE TERRAIN.

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La bio-accumulation d'aluminium dans les tissus de l'écrevisse, Orconectes virilis, et son élimination ont fait l'object de travaux au milieu de la saison estivale dans la Région des Lacs Expérimentaux (au nord-ouest de l'Ontario).

Des écrevisses provenant d'un lac expérimental non modifié (n°239), avec un taux moyen de 36 ug/L d'Al dans l'eau, ont été placées dans des cages en forme de tubes de 1 mètre de diàmetre dans le lac n° 302 (concentration moyenne en Al: 8 µg/L). On a muni ces tubes d'Al (40 µg/L) ou de Cd (1 ou 3 µg/L) ou on les a immergés comme instruments de mesure, sans y ajouter de métaux. On a acidifié une moitié des tubes de chaque traitement métal à un pH de 5.3; les autres tubes sont restés à un pH de 6.7. Aucune des écrevisses n'a accumulé d'Al dans ses tissus. Les écrevisses maintenues dans les tubes exempts d'Al avaient un taux d'Al inférieur dans la glande digestive et le muscle abdominal après un temps d'exposition de 25 à 27 jours comparé au taux enregistré chez les écrevisses fraîchement capturées. Les écrevisses du tube contenant 40 µg/L d'Al à un pH de 5.3 ont retenu des résidus d'Al dans leur glande digestive mais non dans leur muscle. Les écrevisses du tube à concentration de 40 µg/L d'Al à un pH de 6.7 ont retenu une [Al] dans leur muscle mais non dans leur glande digestive. Etant donné tous les traitements métaux, le pH n'a pas eu une incidence claire sur la [Al] dans les tissus. L'addition de Cd dans certains tubes n'a pas influence la [Al] dans les tissus des écrevisses.

Au cours d'une expérience en laboratoire, on a mesuré le taux d'Al dans les tissus des trois groupes d'écrevisses provenant du lac n° 239. Celles du premier groupe ont été disséquées quelques heures après avoir été capturées pour déceler la présence d'Al dans six tissus avant traitement. Celles du deuxième groupe ont été disséquées à la suite d'une exposition en laboratoire à un taux d'Al de 500 µg/L pendant 14 jours. Aucun des tissus examinés n'a indiqué une augmentation de la [Al]. Celles du troisième groupe ont été exposées à un taux d'Al de 500 µg/L pendant 14 jours, et ensuite maintenues dans l'eau du lac n° 239 pour une période supplémentaire de 16 jours. On n'a pas nourri les écrevisses pendant l'expérience. Après l'exposition de 30 jours, seules les carapaces et les branchies comportaient des résidues d'Al. Tous les autres tissus indiquaient une [Al] inférieure au bout des 30 jours. La [Al] totale de l'eau ne semble pas influencer sur la [Al] dans les tissus. Les constations des ces expériences ont donné lieu à l'hypothèse actuellement vérifiée que les baies constitueraient l'emplacement les plus important où l'écrevisse absorbe de l'Al, que ce soit par le biais du régime alimentaire ou par l'ingestion accidentelle de matière non organique.

INTRODUCTION

Acidified freshwaters frequently have higher concentrations of aluminum than circum-neutral waters (Dickson 1978; Cronan and Schofield 1979; Wright et al. 1980; Wright and Skogheim 1983). Toxic effects of Al and the dependence of toxicity on pH, particularly in fish, have been described (Spry et al. 1981; Baker and Schofield 1982). Nevertheless, few studies have been made on the [Al] in aquatic organisms and factors which affect it. We describe two experiments which measured changes in Al content of several tissues of the crayfish, Orconectes virilis, held in the field and in the laboratory.

The purpose of the field experiment was to test the hypothesis that metals (Al and Cd) are accumulated more rapidly by organisms in acidic than in circum-neutral conditions. Experimental enclosures were used to isolate aquatic communities including crayfish in a Precambrian Shield lake. Acid, Al and Cd were added to some of the enclosures. We describe the results of analyzing tissues of the crayfish for Al after they were held 25 to 27 days in cages in the enclosures. Tissues of freshly-collected crayfish were analyzed for comparison. The effects of acid and two Cd concentrations on the [Cd] in the tissues of the crayfish will be published elsewhere.

The purpose of the laboratory experiment was to test the hypothesis that Al in crayfish tissue is relatively labile. An attempt was made to load the tissues of crayfish with Al by exposing them to a high Al concentration (500 µg/L), then observe whether the Al content of tissues returned to background levels after two weeks exposure to unmodified lake water.

MATERIALS AND METHODS

Description of lakes

The enclosure experiment was performed in Lake 302 of the Experimental Lakes Area (ELA), northwestern Ontario. This lake is described by Chang et al. (1983).

Since crayfish are not abundant in Lake 302 they were captured from Lake 239 for the experiment. Lake 239 is approximately 1.5 km from Lake 302 at 49°39'30"N latitude and 93°44'W longitude. Lake morphometry is described by Brunskill and Schindler (1971). Lake 239 has not been modified experimentally but has been affected by forest fire (Schindler et al. 1980). Both lakes have water chemistry typical of Precambrian Shield lakes with conductivity between 20 and 35 µS/cm and pH of 6.6 to 7.3 (Table 1). Cadmium concentration in ELA is generally below 0.5 µg/L, the present analytical limits of detection.

Tube experiment in Lake 302

Fourteen experimental enclosures or tubes, 1 m in diameter and 2 to 3 m in height, were positioned in the epilimnion of the south end of the south basin of Lake 302 on 27 May 1981. Tube structure and position in the lake were similar to those used in 1980 (Chang et al. 1983). The tubes were anchored into the sediment which formed the bottom of the tube.

Table 3. Concentration of Al (μg/g wt weight) in eight tissues from crayfish held in 500 μg/L Al for 14 days, from crayfish held in 500 μg/L Al for 14 days then tap water for 16 days and from 10 crayfish freshly collected from Lake 239. Car=carapace; hep=hepatopancreas; gr gl=green gland; mus=abdominal muscle. Values are $\bar{X}$±S.E. or actual data from pooled analyses. The F statistic from one-way analysis of variance is given and the probability (P) of no differences.

		Tissues							
		car	hep	gr gl	gills	gut	ovary	mus	rest of body
Freshly collected	No. of crayfish	10	10	10	10	10	3	10	10
	No. of chemical analyses	4	5	1	3	1	1	10	10
	Mean Al conc.	65.2± 4.1	19.6±4.0	84.4	32.9±7.1	774	50.4	6.1±1.6	71.7±12.1
Held in 500 μg/L Al	No. of crayfish	9	9	9	9	8	3	9	9
	No. of chemical analyses	9	9	9	9	8	3	9	9
	Mean Al conc.	46.9±14.7	9.1±1.5	105.6±30.4	38.3±4.9	89.0±24.6	<17,27.4,<11	4.4±0.9	24.0± 4.3
Held in 500 μg/L Al then tap water	No. of crayfish	10	10	10	10	10	2	9	10
	No. of chemical analyses	2	2	1	1	1	1	9	10
	Mean Al conc.	51.2± 6.2	2.35±0.3	1.1	21.0	16.0	<1.0	<1.0	31.8± 5.7
	ANOVA F	1.29	7.36		0.26				10.2
	P	>0.05	<0.01		>0.05				<0.001

To test for water-tightness, NaCl (5.08 g) was added to each tube on 17 June. This raised the concentration of Na^+ in tube water from 0.67 mg/L to approximately 1.7 mg/L. The decline of $[Na^+]$ in the tubes indicated that the half time for water renewal in the 14 tubes varied from 8 to 61 d and averaged 29.4 ± 4.4 days ($\overline{X}$ ± SE).

On 17 June half of the tubes were acidified with H_2SO_4 to lower the pH from 6.7 to a target of 5.3. The pH of the acidified tubes was measured and adjusted as necessary every one or two days thereafter. No adjustment of pH was made in the circum-neutral tubes (pH 6.7). On 23 June, 50 ml of an Al solution prepared from $AlCl_3 \cdot 6H_2O$ (14.32 g/L) were added to two tubes to increase their concentrations by 40 μg/L. These tubes were designated as pH 6.7, 40 μg/L Al and pH 5.3, 40 μg/L Al. On 1 July, a second addition of Al was made to the two Al tubes to bring the [Al] back to the target levels. Cadmium was added on 23 June as $CdSO_4 \cdot H_2O$ to eight tubes (four at pH 5.3, four at pH 6.7) to increase their concentrations by 1 or 3 μg/L Cd. Cadmium was added again on 1 July to return [Cd] to target levels. Four additional tubes served as controls; two were acidified to pH 5.3; two remained at the lake pH (6.7).

Crayfish were placed in the tubes in mesh cages as described by Chang et al. (1983) except that the cages were constructed of plastic mesh (17 mm x 16 mm) to prevent the leaching of metals. To prevent escape of crayfish, the chambers of the cages were lined with nylon mesh of 1.8 x 1.45 mm. Two cages each containing five crayfish were placed on the sediment surface in each tube on 2 July.

Collection and body parameters of crayfish

Crayfish were collected from Lake 239 by SCUBA or skin-diving in shallow water at night from 18 to 30 June 1981. They were kept in submerged wire mesh cages in the littoral zone of Lake 239 until used on 2 July. The 140 crayfish used in this experiment averaged 5.14 ± 0.13 g in weight, (range 2.27 to 10.7 g) and 2.75 ± 0.02 cm (range 2.27 to 3.47 cm) in carapace length. Sex ratio (3f:7m) was relatively constant from tube to tube and mean weight was not different among tubes (ANOVA, df 13,126; F = 0.53, P>0.9).

Crayfish were placed into each tube for 25 to 27 days, after which time they were dissected and hepatopancreas, abdominal muscle and the green glands were removed for Al analyses. Twenty crayfish, 9 females and 11 males, were collected on 6 August 1981 from Lake 239 to provide background levels of Al in tissues. They weighed 5.68 ± 0.32 g and the carapace lengths averaged 2.80 ± 0.05 cm.

On 4 July 1983 10 crayfish (3 females, 7 males) were collected from Lake 239 for the determination of background levels of Al. Within hours of capture of crayfish, the following tissues were dissected: carapace, hepatopancreas, green glands, gills, gut and abdominal muscle. These tissues plus the remainder of the body, i.e. ventral part of thorax, exoskeleton of abdomen plus the appendages, were each analyzed for Al. The ovaries from the females in the sample were also analyzed. The average weight of the crayfish was 5.01 ± 0.36 g and carapace length was 2.73 ± 0.05 cm.

Laboratory experiment on loading and depuration of aluminum by crayfish

To observe whether changes in aluminum concentrations in tissues of crayfish can occur relatively rapidly, an attempt was made to load crayfish with Al during a two-week period, then to monitor depuration at the end of the following two-week period.

Crayfish were collected on 1 June 1983 from Lake 239. They were kept in the laboratory in water drawn from the metalimnion of Lake 239, referred to as tap water. Mean composition of tap water from 21 June to 17 July expressed as mg/L (±S.E.) was (N = 8):Na^+ 1.24 ± 0.01; K^+ 0.76 ± 0.02; Mg^{++} 0.94 ± 0.01; Ca^{++} 2.94 ± 0.02. The [Al] in tap water was 23.5 ±1.6 µg/L (N = 5) and pH was 6.7 when freshly drawn and 7.0-7.1 after equilibration with air. Crayfish were fed commercial dogfood in extruded pellet form between 1 June and 7 June. From 7 June, 19 crayfish were placed in 10 L of tap water to which reagent grade $AlCl_3 \cdot 6H_2O$ was added to bring the Al concentration to the target value of 500 µg/L Al. The pH was not adjusted and varied between 6.25 and 7.1. The crayfish were held in a glass aquarium in a Conviron® controlled environment chamber maintained at 16 ± 1.5°C. Photoperiod was set to the natural day-night length for this time of the year. The medium was continuously aerated. Crayfish were not fed during the experiment. The crayfish were exposed to the 500 µg/L Al for 14 days. The medium was renewed every 3 to 4 days. On 29 June, nine of the crayfish were dissected for Al analysis. The remaining 10 crayfish were exposed to unmodified tap water from 30 June to 4 July at which time they were dissected for Al analysis. Water was renewed every 3 days. The pH was not adjusted and varied between 6.05 and 6.50.

Analysis of aluminum in tissues and water

Aluminum in tissues was analyzed by the Freshwater Institute Analytical Laboratory following wet digestion with nitric perchloric acid in Teflon beakers. In many cases, tissues from more than one animal were pooled in order to obtain sufficient tissue weight for the analysis. Aluminum in tissues and in lake water was determined by emission spectrometry with slight modification (A. Lutz, pers. comm.) of the method of Johnson et al. (1979). Using National Bureau of Standards certified reference material, i.e. citrus leaves, this method gave a result of 85.17 ± 13.50 µg Al/g leaves ($\overline{X}$ ± S.D.) N = 15. The certified value for this material was 92 ± 15 µg Al/g (R. Hunt, pers. comm.).

During the laboratory experiment in 1983, reactive Al in water was determined by the lumogallion method of Hydes and Liss (1976) as used by Playle et al. (1982). Water samples were collected in polyethylene acid-washed bottles and stored in a refrigerator or until analysis within 48 h. Samples and freshly-prepared standards were heated in a water bath for 1 h at 80°C and cooled to room temperature before analysis. The fluorescence was read on a Turner Model III fluorometer at a wavelength of 555 nm. Standards were prepared from reagent grade $AlCl_3 \cdot 6H_2O$.

Statistical tests

Statistical significance of differences between metal concentrations in freshly-collected crayfish and those in tube-held crayfish were shown by the exclusion of the latter mean from the 95 or 99% confidence interval around the mean concentrations from the freshly-collected crayfish (Steel and Torrie 1960). The effects of metal (no metal addition, 40 μg/L Al, 1 μg/L Cd or 3 μg/L Cd) and pH (6.7 or 5.3) on concentrations of Al in the crayfish from tubes were determined by a two-way analysis of variance (unbalanced design) (Hewlett-Packard Statistical Library for Series 200 computers) followed by Student-Newman-Keuls' test between pairs of treatments (Steel and Torrie 1960). Significance was accepted at the $P=0.05$ level.

Aluminum concentrations in tissues from the laboratory experiment were compared by one-way analysis of variance following log (X + 1) transformation to reduce inequality of variance. Single [Al] resulting from pooling of tissues within a treatment were compared to [Al] from other treatments by exclusion from their 95% confidence interval.

Unless otherwise stated, variability around means is expressed as standard error.

RESULTS

Concentrations of aluminum in tissues from freshly-collected and tube-held crayfish

Of 140 crayfish introduced into cages in 14 tubes in Lake 302 on 2 July, 1981, 94 or 67% were recovered alive on 27 to 29 July. Thus, one-third of the crayfish introduced into the tubes either died (37 individuals) or escaped (9 individuals). Of the crayfish recovered alive at the end of the experiment, 19% had molted. Of those which died during the experiment, 30% succumbed while molting.

Wet weights of hepatopancreas, abdominal muscle and green gland in 20 crayfish collected on 6 August for background [Al] averaged 5.1%, 10.7% and 0.36%, respectively, of whole body wet weights.

Hepatopancreas

Background [Al] in the hepatopancreas of these crayfish dissected within hours of collection from Lake 239 on 6 August 1981 ranged from 2.8 to 14.0 μg/g tissue wet weight, with a mean and 99% confidence interval of 3.71 ± 1.47 μg/g (Table 2). Crayfish held in the control tubes (no metal addition) for 25 to 27 days had a mean [Al] in the hepatopancreas outside the confidence interval around hepatopancreas [Al] in the freshly-collected crayfish (Table 2). Furthermore, mean hepatopancreas [Al] in crayfish from each tube, except for the 40 μg/L, pH 5.3 tube was lower than in the freshly-collected crayfish ($P<0.01$). Mean hepatopancreas [Al] in the crayfish from the 40 μg/L Al, pH 5.3 tube was not different from that in the freshly-collected crayfish ($P>0.05$).

Among the 140 crayfish held in the 14 tubes, hepatopancreas [Al] was

Table 2. Concentration of Al (μg/g wt weight) in three tissues from crayfish held in tubes for 25-27 days in Lake 302 and from 20 crayfish freshly collected from Lake 239 in 1981. Hep = hepatopancreas; mus = abdominal muscle; gr gl = green gland. Values are $\overline{X}$ ± S.E. or actual data from pooled analyses.

Experimental conditions	No. of crayfish			No. of chemical analyses after pooling of tissues			[Al] in tissues		
	hep	mus	gr gl	hep	mus	gr gl	hep	mus	gr gl
Freshly-collected	20	19	20	16	15	2	3.71 ± 0.50	2.09 ± 0.34	39.8, 1.7
Tube held:									
no metal addition pH 6.7	14	14	14	2	2	1	0.98, 0.21	1.46, 0.67	4.63
no metal addition pH 5.3	10	10	10	2	2	1	1.53, 0.78	0.56, 0.24	1.88
40 μg/L Al pH 6.7	9	9	14	1	1	1	1.51	1.59	2.99
40 μg/L Al pH 5.3	5	5	pooled with 40 μg/L Al pH 6.7	1	1	0	3.23	0.95	—
1 μg/L Cd pH 6.7	15	15	15	2	2	1	1.76, 1.42	0.77, 0.26	0.44
1 μg/L Cd pH 5.3	13	13	13	2	2	1	1.13, 1.27	1.27, 0.34	1.10
3 μg/L Cd pH 6.7	12	12	12	2	2	1	1.10, 1.09	0.51, 0.17	5.71
3 μg/L Cd pH 5.3	16	16	16	2	2	1	1.15, 0.88	0.71, 0.80	1.15

different among the four metal treatments (no metal addition, 40 µg/L Al, 1 µg/L Cd, 3 µg/L Cd) (two way ANOVA df 1, 3; F=9.6; P=0.010) but not between tubes of different pH (6.7 vs 5.3) (df 3, 1; F=2.3; P=0.183). The crayfish from tubes receiving Al had hepatopancreas [Al] higher than those from the control and Cd tubes (Student-Newman-Keuls' test, $P<0.05$). The crayfish from the control and Cd tubes were not different from one another in hepatopancreas [Al] concentration (Student-Newman-Keuls' test, $P>0.05$). Although there was no overall influence of pH on [Al] of hepatopancreas among the 14 tubes, the mean [Al] in hepatopancreas of 10 crayfish in the 40 µg/L Al, pH 5.3 tube (3.23 µg/g) was twice as high as in the 40 µg/L Al, pH 6.7 tube (1.51 µg/g). Applying the 99% confidence interval from the chemical analyses of [Al] in freshly-collected crayfish (± 1.47 µg/L) to 1.51 µg/g wet weight [Al] in hepatopancreas from the 40 µg/L Al, pH 6.7 tubes indicates that [Al] in hepatopancreas from the 40 µg/L Al, pH 5.3 tube was significantly higher ($P<0.01$). Therefore, for crayfish in the Al-enriched tubes only, low pH resulted in significantly higher Al content in hepatopancreas compared with circum-neutral pH. None of the differences in hepatopancreas [Al] between control or Cd-enriched tubes of different pH were as large as ± 1.47 µg/g.

Abdominal muscle

The abdominal muscle of the freshly-captured crayfish from Lake 239 contained from 0.55 to 4.05 µg Al/g wet tissue weight, with a mean and 99% confidence interval of 2.09 ± 1.01 µg/g (Table 2). Mean [Al] in muscle from crayfish in each tube was significantly lower than that in freshly-collected crayfish (using the confidence interval of 2.08 ± 1.01 µg/g) except for that from the 40 µg/L Al, pH 6.7 tube. Two way unbalanced design ANOVA performed on the mean muscle [Al] among crayfish from the 14 tubes indicated no effect of pH (F=0.14, P=0.72) or of metal (F=1.49, P=0.31). The confidence interval from the freshly-collected crayfish (± 1.01 µg/g) applied to the mean muscle [Al] from tube-held crayfish indicated no differences in any of the four treatments between tubes of different pH. Concentrations of Al in the abdominal muscle were significantly less than those in the hepatopancreas both in the crayfish sampled from the tubes (ANOVA, F=6.5, $P<0.025$) and in the freshly collected crayfish (ANOVA, F=7.0, $P<0.025$).

Green gland

The tissue containing the highest but most variable [Al] was the green gland (Table 2). Because of the small size of this tissue, pooling from individual crayfish occurred to a greater extent than for other tissues, resulting in fewer chemical analyses. There were no clear differences among tube-held crayfish in [Al] in the green gland between freshly-collected and tube-held crayfish.

In summary, in none of the tubes did crayfish accumulate Al in their tissues above background levels. Instead, in nearly all tubes, crayfish lost Al from hepatopancreas and abdominal muscle. Only crayfish from 40 µg/L Al, pH 5.3 retained background [Al] in hepatopancreas and crayfish from 40 µg/L Al, pH 6.7 retained background [Al] in abdominal muscle.

Concentration of alumunium in the tubes and in Lake 302

Time-weighted average of [Al] in the 40 µg/L, pH 6.7 tube in Lake 302 between 2 and 27 July was 42.03 µg/L. For the 40 µg/L, pH 5.3 tube, the time-weighted average was 59.35 µg/L. Average [Al] on 27 July for the non-Al tubes at pH 6.7 was 16.8 ± 1.3 µg/L and for the six non-Al tubes at pH 5.3 was 22.2 ± 1.4 µg/L. These latter concentrations were significantly different (ANOVA, F=7.8, $P<0.025$).

Although the ice-free season mean [Al] of the epilimnion, based on sampling Lake 302 South at the centre, was 8.09 µg/L in 1981, [Al] in the vicinity of the tubes was 21.2 µg/L based on 4 samples between 29 May and 5 Aug (time weighted average).

Loading and depuration experiment in the laboratory

Background [Al] in the 10 crayfish from Lake 239 analyzed in 1983 was highest in the gut (774 µg Al/g wet tissue weight). The abdominal muscle contained the lowest [Al], 6.1 ± 1.6 µg/g wet weight (Table 3). The other tissues were intermediate in [Al] and in decreasing order of concentration were: green gland > remainder of body > carapace > ovary > gills > hepatopancreas (Table 3). As percentages of body weight in the 10 crayfish, these parts averaged: carapace, 4.39%; hepatopancreas, 6.71%; green glands, 1.70%; gills, 3.21%; gut (proventriculus and intestine), 1.26%; ovary, 0.81%; abdominal muscle, 12.06% and remainder of body, 55.83%. For the 29 crayfish in the laboratory experiment, sum of wet weights of tissues averaged 80.3% of live weights.

Nine crayfish showed no accumulation of Al after 14 days exposure to 500 µg/L Al. Analysis of variance on transformed data [log (X + 1)] comparing tissue [Al] of freshly-collected crayfish with those exposed only to 500 µg/L Al for 14 d and with those exposed to 500 µg/L Al for 14 d then to tap water for 16 d indicated no difference between the three treatments for carapace (df 2,1; F=1.29; P=0.31). Concentration of Al in the carapace remained between 45 and 65 µg/g wet weight (Table 3). The [Al] in hepatopancreas, in contrast, was highly different between treatments (df 2, 13; F=7.36, P=0.007). Hepatopancreas [Al] was significantly reduced from the fresh level of 19.6 µg/g weight weight to 9.1 µg/g after exposure to 500 µg/L Al (Table 3) (Student-Newman-Keuls' test, $P<0.05$), but the tissue [Al] after the additional exposure to tap water (2.35 µg/g) was not significantly lower (Student-Newman-Keuls' test, $P>0.05$). Because of the small number of chemical analyses on green gland for two of the treatments, the [Al] of freshly-collected crayfish and those after the 500 µg/g Al and the tap water exposure were compared with the mean and 95% confidence interval (105.6 ± 70.1 µg/g) around the [Al] in crayfish exposed only to 500 µg/L Al. This test showed that green gland was not lower than freshly-collected crayfish in [Al] after exposure to 500 µg/L Al but lost most of its [Al] after the 16 d tap water exposure (Table 3). The [Al] of gills did not change with treatment (ANOVA on transformed data, df 1,10; F = 0.26, P = 0.62), (Table 3). The effect of treatment on [Al] in gut was compared as for green gland. The 95% confidence interval around the gut [Al] of 500 µg/L Al-exposed crayfish was 89.0 ± 58.2 µg/g. Therefore, compared with freshly-collected crayfish the gut lost Al after exposure to 500 µg/L and showed an additional loss after the tap water

Table 3. Concentration of Al (µg/g wt weight) in eight tissues from crayfish held in 500 µg/L Al for 14 days, from crayfish held in 500 µg/L Al for 14 days then tap water for 16 days and from 10 crayfish freshly collected from Lake 239. Car=carapace; hep=hepatopancreas; gr gl=green gland; mus=abdominal muscle. Values are $\bar{X}$±S.E. or actual data from pooled analyses. The F statistic from one-way analysis of variance is given and the probability (P) of no differences.

					Tissues				
		car	hep	gr gl	gills	gut	ovary	mus	rest of body
Freshly collected	No. of crayfish	10	10	10	10	10	3	10	10
	No. of chemical analyses	4	5	1	3	1	1	10	10
	Mean Al conc.	65.2± 4.1	19.6±4.0	84.4	32.9±7.1	774	50.4	6.1±1.6	71.7±12.1
Held in 500 µg/L Al	No. of crayfish	9	9	9	9	8	3	9	9
	No. of chemical analyses	9	9	9	9	8	3	9	9
	Mean Al conc.	46.9±14.7	9.1±1.5	105.6±30.4	38.3±4.9	89.0±24.6	<17,27.4,<11	4.4±0.9	24.0± 4.3
Held in 500 µg/L Al then tap water	No. of crayfish	10	10	10	10	10	2	9	10
	No. of chemical analyses	2	2	1	1	1	1	9	10
	Mean Al conc.	51.2± 6.2	2.35±0.3	1.1	21.0	16.0	<1.0	<1.0	31.8± 5.7
	ANOVA F	1.29	7.36		0.26				10.2
	P	>0.05	<0.01		>0.05				<0.001

exposure.

Statistical analyses were not performed on [Al] of ovary because of the imprecision of the values. Nevertheless, the values do not overlap and the ovary lost Al with each successive treatment. Abdominal muscle did not change in [Al] from freshly-collected values with exposure to 500 µg/L Al (ANOVA on transformed data, df=1, 18; F=0.41, P=0.53). The additional exposure to tap water resulted in a loss of Al in muscle which is obvious without statistical testing. In each of nine crayfish the muscle contained <1.0 µg/g Al after the tap water exposure (Table 3). The remainder of the body, comprising over half the crayfish live weight, varied in [Al] with treatment (ANOVA on transformed data df 2, 26; F=10.2, P=0.0005). The exposure to 500 µg/L resulted in a significant loss of Al compared with freshly-collected concentrations but there was no further loss after the tap water exposure (Student-Newman-Kuels' test, P<0.01). In summary, over the 30 d exposures, only the carapace and gills retained their Al content. All other tissues had lower [Al] at the end of the 30 d.

Figure 1 shows that the [Al] in the holding water during the exposure to 500 µg/L Al fell over the 3 or 4 days the crayfish were held between changes of water. Presumably this is due to adsorption to the glass or the crayfish

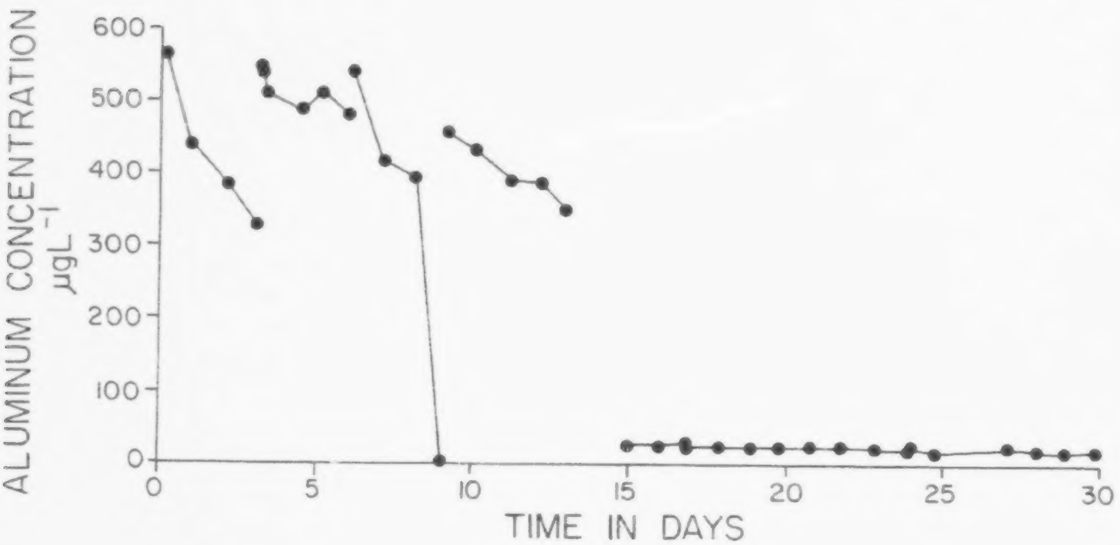

Fig. 1 Observed aluminum concentrations in the medium of crayfish exposed in the laboratory first to 14 days of target Al concentration of 500 µg/L followed by 16 days to tap water.

or precipitation of Al from this supersaturated "solution". On about day 8 of the experiment, some crayfish molted and plastic mesh containers were placed in the aquarium to provide shelter. Clearly these containers strongly adsorbed Al and on day 9 [Al] was below detection in the medium. This did not significantly reduce the overall exposure of the crayfish to Al which on the average during the 14 days was above 400 μg/L.

DISCUSSION

We tested the hypothesis that Al is accumulated more rapidly by crayfish in acidic than in circum-neutral conditions. Unexpectedly, we found that Al was not accumulated by crayfish in Al-enriched conditions in either pH 6.7 or 5.3. In fact, Al was lost from the hepatopancreas in the 40 μg/L, pH 6.7 tubes and from the abdominal muscle in the 40 μg/L, pH 5.3 tube. Only muscle in the former tube and the hepatopancreas in the latter tube retained background [Al]. Crayfish from all the non-Al enriched tubes lost Al from both tissues. No overall effect of pH on [Al] in crayfish tissues was seen in this experiment. The presence of Cd in some tubes had no measurable effect on [Al] in crayfish tissues.

This experiment was designed to expose the aquatic community (zooplankton and algae) of Lake 302 to about a four-fold higher (40 μg/L) than normal [Al]. Ice-free season mean [Al] measured at the centre of the south basin of Lake 302 were 6.31, 8.09 and 13.0 μg/L, respectively, in 1980, 1981 and 1982 (D. Cruikshank, pers. comm.). Nevertheless, the crayfish were transferred to the 40 μg/L Al tubes from a lake, 239, with background levels of Al higher than those in Lake 302 (Table 1). Therefore, the crayfish experienced a smaller enrichment in [Al] of about 1.5 to 2-fold. Lake 239 crayfish transferred to the non-Al tubes were exposed to [Al] lower than in their habitat. This remained true even though [Al] concentration in the non-Al tubes rose with time as indicated above to 16 to 22 μg/L. Increase in [Al] with time in control tubes was also seen in the tube experiment in Lake 302 in 1980 described by Chang et al. (1983). Increase in [Al] outside the tubes in the bay was also seen in midsummer, 21 μg/L Al, compared with the ice-free season whole-lake average of 8 μg/L in 1981. Even though several factors tended to elevate the [Al] of non-Al tubes, the crayfish in these tubes were still exposed to slightly lower [Al] than in Lake 239 and possibly lost Al because of the reduced external [Al]. Nevertheless, the crayfish in the Al-enriched tubes were expected to take up Al. Instead, the experiment revealed that Al is a fairly mobile metal in crayfish and was lost relatively easily from both hepatopancreas and abdominal muscle even in Al enriched conditions.

This led to the attempt to demonstrate the lability of Al by loading crayfish with Al from an environment supersaturated with Al over a two week period followed by measurement of the loss of the accumulated Al after a two-week exposure to tap water. The attempt to load crayfish with Al in the laboratory was not successful. Crayfish exposed to the supersaturated 500 μg/L Al for 14 days not only did not take up Al but lost it from the body. Subsequent exposure to tap water which was obtained from the same lake as the crayfish produced further losses of Al from tissues.

It became clear that the [Al] of the external environment was not directly controlling [Al] in tissues. The hypothesis most consistent with the laboratory results is that the primary source of Al to crayfish is through feeding. Were the primary source to be from the water via the gills, it would be possible to explain the loss of Al in the non-Al tube-held crayfish from exposure of crayfish to a lowered [Al] in the water. But the laboratory-held crayfish would have been expected to at least maintain their [Al] since they were in the same water as in their natural habitat. This loss of Al implicates feeding as the primary source of Al, probably from the food or possibly from sediment incidentally ingested.

In the tube experiment, crayfish were caged and unable to feed completely normally. The crayfish in the Al-enriched tubes (at least in the 40 µg/L, pH 5.3), were able to maintain Al in the hepatopancreas. Presumably the crayfish were exposed to the added Al through some feeding by virtue of the proximity of the cages to the sediment.

It is suggested, therefore, that field experiments examining the factors which affect bioaccumulation of Al by crayfish allow the animals to feed normally and that laboratory experiments be conducted to examine food as a source of Al to crayfish.

The background concentrations of Al measured in tissues from two groups of control crayfish varied more than expected. Twenty crayfish collected on 6 August 1981 had mean [Al] of 3.7 and 2.1 µg Al/g wet tissue weight in hepatopancreas and abdominal muscle, respectively. Green gland concentrations were variable, 39.8 and 1.7 µg/g in two pooled tissue samples. The background concentration in 10 crayfish collected on 4 July 1983 were five-fold higher for hepatopancreas ($P<0.01$) and three-fold higher for abdominal muscle ($P<0.05$). The crayfish in 1983 had a pooled green gland [Al] of 84.4 µg/g. The reason for the difference between years is not known but it is not believed to be due to differences in mean size or sex ratio in the crayfish, or to any change in the Al content of Lake 239. There was no relationship between hepatopancreas [Al] and body weight ($r=0.28$; $P>0.5$) or abdominal muscle [Al] and body weight ($r=0.45$; $P>0.1$). The possibility of a seasonal change between July and August in Al content in crayfish in Lake 239 has not been examined but considered unlikely. A final possibility is that the crayfish were collected from different locations in Lake 239 which differed in bioavailability of Al. Locations of the collections within the lake were not recorded.

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EFFECTS OF ACID WATER ON THE TOXICITIES OF CADMIUM, COPPER AND ZINC TO TROUT

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Increased metal concentrations have been associated with fresh water acidification. Continuous-flow acute toxicity tests were conducted in very soft water (10 mg/L $CaCO_3$) to determine the effect of pH on the toxicity of cadmium, copper and zinc to small (1-6g) steelhead trout (*Salmo gairdneri*). Calculations of LC_{50} values were made for 96 and 168 h exposure periods in waters of pH 4.7, 5.7 and 7.0. Fish were acclimated to test pH for six days in outdoor holding tanks, transferred to the testing gear and held for two more days prior to testing. The test fish were significantly more tolerant of the metals at the lowest pH value than at higher pH's. The 96 h LC_{50} values for zinc at the three pH values were 671, 97 and 66 µg/L. The values were 66.0, 4.2 and 2.8 µg/L for copper, and 28.0, 0.7 and <0.5 for cadmium. The 168 h values were similar to the 96 h values.

The results of this experiment indicate that for the metals tested, in contrast to aluminum, toxicity is ameliorated in depressed pH waters over short exposure periods, such as may occur during snowmelt runoff. The possibility of hydrogen ion interference with metal uptake is postulated.

LES EFFETS DE L'EAU ACIDE SUR LA TOXICITÉ DU CADMIUM, DU CUIVRE ET DU ZINC AUX TRUITES

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L'augmentation des concentrations de métaux a été associée avec l'acidification de l'eau douce. Des analyses de toxicité aiguë en courant continu furent conduites dans des eaux très douces (10 mg/L de $CaCO_3$) pour déterminer les effets du pH sur la toxicité du cadmium, du cuivre et du zinc à des petites (1-6 g) truites arc-en-ciel (Salmo gairdneri). Les calculations des valeurs de CL_{50} furent faites pour des périodes d'exposition de 96 et de 168 heures dans des eaux à pH de 4.7, 5.7 et 7.0. Les poissons furent acclimatés au pH testé, pour 6 jours, dans des réservoirs en plein air. Ils furent ensuite transférés à l'appareillage expérimental et gardés pour 2 jours avant le début des analyses. Les poissons testés furent significativement plus tolérants aux métaux aux valeurs basses de pH qu'aux hauts pH. Les valeurs du CL_{50} de 96 h pour le zinc aux trois pH étaient de 671, 97 et 66 µg/L. Les valeurs furent de 66.0, 4.2 et 2.8 µg/L pour le cuivre, et de 28.0, 0.7 et <0.5 µg/L pour le cadmium. Les valeurs pour 168 h étaient similaires aux valeurs trouvées pour 96 h.

Les résultats de cette expérience indiquent que pour les métaux analysés, contrairement à l'aluminum, la toxicité est améliorée dans les eaux avec des pH amoindris pour de courtes périodes d'exposition, comme durant les écoulements de la fonte des neiges. La possibilité d'interférence des ions d'hydrogène avec l'absorption des métaux est postulée.

ALUMINUM UPTAKE AND TOXICITY TO DAPHNIA MAGNA IN SOFT WATER AT LOW PH

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The rate of aluminum uptake and the effect of aluminum on mortality and on the sodium, chloride and calcium content of *Daphnia magna* were determined. Laboratory experiments were conducted at 3 concentrations of aluminum (0, 0.3, 1.0 mg/L) in soft water (2.5 and 12.5 mg Ca/L) adjusted to pH 4.5, 5.0 and 6.5 (control).

The results showed that aluminum toxicity as well as aluminum uptake were both pH dependent with maximum toxicity and maximum uptake (630 mM within 48 h) at pH 6.5. There was also some mortality ascribable to aluminum at pH 5.0, while at pH 4.5 aluminum reduced hydrogen ion toxicity.

Mortality, in the presence of aluminum and also at low pH, was associated with a decrease in the sodium and chloride content of daphnids. At pH 5.0 with equimolar concentrations of aluminum and calcium in the water, there was a significant decrease in total body calcium content, which was negatively correlated with aluminum content.

Calcium additions temporarily reduced both aluminum and hydrogen ion toxicity, reduced the net loss of sodium and chloride, but had no effect on aluminum uptake.

Aluminum and hydrogen ions affect salt regulation in *D. magna*. Small additions of calcium to the water can reduce the rate of mortality as well as the rate of net sodium and chloride loss. Uptake of aluminum occurs rapidly at circumneutral pH and may be an additional source for fishes and other predatory organisms.

L'ABSORPTION D'ALUMINIUM ET SA TOXICITÉ ENVERS *DAPHNIA MAGNA* DANS DE L'EAU DOUCE À BAS PH

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On a déterminé la vitesse d'absorption et l'effet de l'aluminium sur la mortalité et sur le contenu en sodium, chlore et calcium des *Daphnia magna*. Des expériences en laboratoire furent conduites avec 3 concentrations d'aluminium (0, 0.3, 1.0 mg/L) en eau douce (2.5 et 12.5 mg Ca/L) ajustée à des pH de 4.5, 5.0 et 6.5 (témoin).

Les résultats montrèrent que la toxicité et l'absorption d'aluminium étaient tous deux dépendants du pH, avec des maxima de toxicité et d'absorption (630 mM en dedans de 48 h) à un pH de 6.5. Il y eut aussi une certaine mortalité attribuable à l'aluminium à un pH de 5.0, tandis qu'à un pH de 4.5 l'aluminium diminuait la toxicité de l'ion hydrogène.

La mortalité en présence de l'aluminium et à bas pH fut associée à une diminution du contenu de sodium et de chlore dans les daphnies. À un pH de 5.0 avec des concentrations équimolaires d'aluminium et de calcium dans l'eau, il y avait une diminution considérable dans le contenu corporel total de calcium, lequel était corrélé négativement avec le contenu en aluminium.

L'addition de calcium diminuait temporairement les effets toxiques de l'aluminium et de l'ion hydrogène, réduisait la perte nette en sodium et en chlorure, mais n'avait pas d'effet sur l'absorption d'aluminium.

Les ions d'aluminium et d'hydrogène affectent la régulation du sel des *Daphnia magna*. De petites quantités de calcium ajoutées à l'eau peuvent diminuer le taux de mortalité ainsi que les taux de perte nette de sodium et de chlore. L'absorption d'aluminium a lieu rapidement à pH neutre et peut constituer une ressource additionnelle pour les poissons et autres organismes prédateurs.

THE EFFECT OF PH AND/OR CALCIUM-ENRICHED FRESHWATER ON GILL CA^{+2}-ATPASE ACTIVITY AND OSMOTIC WATER INFLOW IN RAINBOW TROUT (*SALMO GAIRDNERI*)

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Rainbow trout (*Salmo gairdneri*) were exposed to control and calcium-enriched freshwater at pH 5.0 and 6.6 for 14 days. Hematocrit, gill Ca^{+2}-ATPase enzyme activity, *in vitro* gill osmotic water inflow, plasma calcium and osmolarity were measured.

No significant changes in hematocrit or plasma calcium ion levels were observed. Plasma osmolarity decreased in fish exposed to calcium-enriched water (60 mg Ca^{+2}/L) relative to those held in 2.0 mg Ca^{+2}/L waters, irrespective of the pH level.

Gill Ca^{+2}-ATPase enzyme activities were measured for both low affinity (3 mM Ca^{+2}) and high affinity (100 M Ca^{+2}) activity. Exposure of rainbow trout to low pH (5.0) did not affect the specific activity of Ca^{+2}-ATPase enzyme. However, low affinity Ca^{+2}-ATPase activity in fish exposed to calcium-enriched water did show a significant reduction. A reduction in Ca^{+2} efflux after acclimation to high external Ca^{+2} concentration may reduce the requirement for active transport of Ca^{+2} into the fish.

Since ionic problems in fish are also related to their permeability to water, the effects of low pH on ionic regulation may be partially due to changes in osmotic permeability. The *in vitro* osmotic water uptake of the gills of rainbow trout were measured after *in vivo* exposure of the fish to the above conditions. The rate of weight increase of the gills did not differ between fish exposed to pH 5.0 and 6.6. Exposure of fish to calcium-enriched freshwater significantly enhanced the rate of osmotic water uptake, irrespective of the pH level. These results are in contrast to other studies that show increasing the ambient Ca^{+2} concentration reduces the osmotic and ionic permeabilities of fish gills. This increase in osmotic water uptake by the gills of fish exposed to an increased calcium environment, reported in the present study, is interpreted as a result of the higher osmolarity of the calcium-enriched media.

L'EFFET DU PH ET/OU DE L'EAU DOUCE ENRICHIE DE CALCIUM SUR L'ACTIVITÉ DE LA Ca^{+2}-ATPASE DANS LES BRANCHIES ET SUR L'AFFLUX OSMOTIQUE D'EAU DANS LA TRUITE ARC-EN-CIEL (*SALMO GAIRDNERI*)

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Des truites arc-en-ciel (*Salmo gairdneri*) furent exposées à de l'eau douce témoin et enrichie de calcium à des pH de 5.0 et 6.0 durant 14 jours. On mesura l'hématocrite, l'activité de l'enzyme Ca^{+2}-atpase des branchies, l'afflux osmotique d'eau dans les branchies *in vitro*, le calcium du plasma et l'osmolarité.

On n'observa aucun changement significatif dans l'hématocrite ou dans les niveaux d'ions de calcium. L'osmolarité du plasma diminua dans les poissons exposés à l'eau douce enrichie de calcium (60 mg Ca^{+2}/L) relativement à ceux tenus dans de l'eau avec 2.0 mg Ca^{+2}/L, et ceci irrespectivement du niveau de pH.

On mesura l'activité de l'enzyme ATPase-Ca^{+2} dans les branchies pour deux types d'affinité: basse (3 mM Ca^{+2}) et haute (100 mM Ca^{+2}). L'exposition des truites arc-en-ciel à un bas pH (5.0) n'affecta pas l'activité spécifique de l'enzyme ATPase-Ca^{+2}. Cependant, l'activité de l'ATPase-Ca^{+2} à basse affinité dans les poissons exposés à l'eau enrichie de calcium, subit une diminution significative. Une réduction dans l'efflux de Ca^{+2} après acclimatation à une haute concentration externe de Ca^{+2} peut diminuer le besoin pour le transport actif du Ca^{+2} dans le poisson.

Etant donné que les problèmes ioniques des poissons sont aussi apparentés à leur perméabilité à l'eau, les effets d'un bas pH sur la régulation ionique peuvent être partiellement dus à des changements dans la perméabilité osmotique. On mesura l'absorption osmotique d'eau *in vitro* dans les branchies de truites arc-en-ciel après les avoir exposées *in vivo* aux conditions ci-haut mentionnées. Le taux d'augmentation de poids des branchies ne différa pas entre les poissons exposés à un pH de 5.0 et ceux exposés à un pH de 6.6. L'exposition des poissons à de l'eau douce enrichie de calcium augmenta d'une façon significative le taux d'absorption osmotique de l'eau, irrespectivement du niveau de pH. Ces résultats contrastent avec ceux d'autres études qui montrent qu'une augmentation de la concentration ambiante de Ca^{+2} diminue les perméabilités osmotique et ionique des branchies de poissons. Cette augmentation dans l'absorption osmotique d'eau par les branchies de poissons exposés à une augmentation de calcium dans l'environnement, telle que rapportée par le présente étude, est interprétée comme étant le résultat de la plus haute pression osmotique du milieu enrichi de calcium.

ALTERATIONS IN THE REPRODUCTIVE CYCLE OF FEMALE BROOK TROUT (SALVELINUS FONTINALIS) IN ACIDIC NORTHERN ONTARIO LAKES

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This study examines aspects of the reproductive physiology of female brook trout in neutral (pH>7.0) and acidic (pH<6.0) lakes. Nine lakes were sampled over three years, with pH ranging from 5.0 to 7.8. Lake size, water temperatures and photoperiod were similar for all lakes, and all were located between 43° - 46° N. Oogenesis was studied by histology and gravimetry; timing of ovulation and spawning were determined by on-site observation. Results indicate that yolky egg development in females from acidic lakes is delayed during July, but by August, no difference could be detected between acid and neutral lakes. In October, acidic lake females were shown to have considerably smaller and lighter ova than neutral lake females, but their fecundity was considerably increased. Ovulation and spawning in neutral lakes occurred in the first and second week of October, whereas in acid lakes, ovulation was first detected in the third week, and spawning in one acid lake may have occurred as late as the second week of November. The resulting aberrations in ovarian development, ovulation and spawning may affect the survival of trout embryos in acidic lakes, and contribute to the decline of lake fish populations. This study was supported by the Ontario Ministry of the Environment.

ALTÉRATIONS DANS LE CYCLE REPRODUCTEUR DE FEMELLES DE TRUITE MOUCHETTÉE (SALVELINUS FONTINALIS) DANS DES LACS ACIDES DU NORD DE L'ONTARIO

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Cette étude examine certains aspects de la physiologie reproductrice de truites mouchetées femelles dans des lacs à pH neutre (pH>7.0) et à pH acide (pH<6.0). Neuf lacs furent échantillonnés sur une période de trois ans, et leur pH variait de 5.0 à 7.8. La grosseur des lacs, la température de l'eau et la photopériode étaient similaires pour tous les lacs, qui étaient tous situés entre 43° et 46°N. L'oogénèse fut étudiée par histologie et par gravimétrie; le temps de l'ovulation et de la ponte fut déterminé par des observations sur place. Les résultats indiquent que le développement des oeufs à embryon de femelles provenant de lacs acides est retardé durant juillet, mais aucune différence ne pouvait être décelée entre les lacs acides et les lacs neutres au mois d'août. Au mois d'octobre, les femelles provenant de lacs acides avaient des ovules considérablement plus petits et plus légers que ceux des femelles de lacs neutres, mais leur fécondité était aussi considérablement plus grande. L'ovulation et la fraye eurent lieu dans la première et la deuxième semaine d'octobre dans les lacs neutres, tandis que dans les lacs acides l'ovulation ne fut détectée que dans la troisième semaine d'octobre, avec la fraye pouvant avoir eu lieu dans un certain lac acide aussi tard que dans la deuxième semaine de novembre. Les aberrations dans le développement des ovaires, dans l'ovulation et dans la fraye peuvent affecter le développement des embryons de truites dans les lacs acides et contribuer au déclin des populations de poissons lacustres. Cette étude fut subventionée par le Ministère de l'Environnement de l'Ontario.

ACIDIFICATION AND TOXICITY OF METALS TO AQUATIC BIOTA

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Prior to about 1970, the effects of pH changes on aquatic organisms were generally interpreted in terms of the physiological response of the organism, with little or no consideration of pH-induced chemical changes in the external medium. In recent years, however, under the pervasive influence of aquatic chemists, the research community has come full circle and the effects of pH changes now tend to be interpreted primarily in terms of speciation changes in the external medium. For metal toxicity, a decrease in pH may reasonably be expected to affect both metal speciation in solution and biological sensitivity at the level of the cell surface. These two responses to acidification are antagonistic and will tend to cancel each other; the observed overall response at the organism level to a pH change from 7 to 4, at constant total metal concentration, may well be positive, negative or null.

In the present study we have reviewed variations in metal bioavailability and toxicity over the pH range 7 → 4. Attention has been focused on some 10 metals of potential toxicity to aquatic biota (Ag, Al, Cd, Co, Cu, Hg, Mn, Ni, Pb, Zn). Emphasis has been accorded not to changes in total metal concentration (i.e. geochemical mobilization), but rather to possible changes in metal speciation occasioned by the decrease in pH and to the concomitant changes in biological susceptibility over this pH range. Within this framework, using in large measure previously published results, we have addressed the following specific questions:

- For which metals will pH-induced changes in speciation be important in the pH range 7 → 4?
- Does the literature provide evidence that the response to metals of aquatic biota is pH dependent? For individual metals, are there any experimentally derived pH-related patterns which agree with those expected theoretically?
- Do producer and consumer organisms respectively differ in their response to pH-metal combinations?
- If low pH does affect metal uptake or toxicity, does this happen at realistic pH and metal combinations, i.e. would there be a practical aspect or is it effectively academic?
- At the present stages of environmental acidification, can one predict which metals, if any, pose a risk to the aquatic biota?

The results of this analysis will be presented.

L'ACIDIFICATION ET LA TOXICITÉ DES MÉTAUX EN RAPPORT AU MILIEU AQUATIQUE

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Avant 1970, les effets des changements de pH sur les organismes aquatiques étaient généralement interprétés en termes de réponses physiologiques par l'organisme, en ne considérant que peu ou pas les changements chimiques induits par le pH dans le milieu externe. Dans les dernières années, cependant, la communauté scientifique est revenue à son point de départ et les changements de pH ont maintenant tendance à être interprétés principalement en termes de changement de spéciation dans le milieu externe. En ce qui a trait à la toxicité des métaux, on peut raisonnablement s'attendre à ce qu'une diminution dans le pH affecte et la spéciation des métaux en solution et la sensitivité biologique au niveau de la surface cellulaire. Ces deux types de réponse à l'acidification sont antagonistes et ont tendance à s'annuler l'une et l'autre; les réponses observées au niveau de l'organisme à un changement de pH de 7 à 4, à une concentration constante de métaux, peuvent être positives, négatives ou nulles.

Dans la présente étude, nous avons réexaminé les variations dans la biodisponibilité des métaux et dans la toxicité à des pH variant de 7 à 4. Nous avons surtout porté attention à quelques 10 métaux de toxicité potentielle au milieu aquatique (Ag, Al, Cd, Co, Cu, Hg, Mn, Ni, Pb et Zn). Nous avons mis l'accent non sur les changements dans la concentration totale des métaux (i.e. mobilisation géochimique), mais plutôt sur les changements possibles dans la spéciation des métaux occasionnée par une diminution de pH et sur les changements concomitants dans la susceptibilité biologique à l'intérieur de cette variation de pH. En utilisant en grande partie des résultats déjà publiés, nous sommes attaqués aux questions suivantes:

- Pour quels métaux les changements de spéciation induits par le pH seront-ils importants entre les pH de 7 à 4?

- Est-ce que la littérature fournit une évidence que la réponse du milieu aquatique aux métaux est dépendante du pH? Pour les métaux individuels, a-t-on expérimentalement dérivé des modèles de comportement en fonction du pH étant en accord avec ceux prédits par la théorie?

- Est-ce que les organismes producteurs et consommateurs diffèrent dans leurs réponses respectives aux combinaisons métal-pH?

- Si le bas pH affecte l'absorption de métaux ou la toxicité, est-ce que ceci arrive à des pH et à des combinaisons de métaux réalistes, i.e. y a-t-il un aspect pratique ou est-ce que ceci n'est qu'une question d'intérêt académique?

- Aux présents niveaux d'acidification de l'environnement, peut-on prédire quels métaux, en assumant qu'il y en ait, posent un risque au milieu aquatique?

Les résultats de cette analyse seront présentés.

FRESHWATER ACIDIFICATION: EFFECTS OF LOW PH ON TRANSFORMATION OF DETRITAL ENERGY BY A SHREDDING CADDISFLY

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Cause and effect relationships underlying the simplification of communities in acidified waters remain largely unknown. The objective of this work was to assess the importance of pH-induced changes in the detritivore-microbe-organic matter component of aquatic food webs as a mechanism of community change.

We hypothesized that elevated acidity reduces the efficiency of energy transfer by shredding invertebrates, directly by increasing shredder maintenance costs and indirectly by reducing nutritional value of the organic matter food source due to inhibited microbial activity.

Rearing of the caddisfly Clistoronia magnifica (Limnephilidae) at low pH did not reduce but rather accelerated larval growth. Larvae reared at pH 4.2 - 5.2 did not only grow faster than larvae reared at pH 5.8 - 6.4 but also produced larger pupae and adults. Studies with ^{14}C-tagged leaf litter indicated no direct effects of pH on partitioning of ingested detrital carbon, suggesting that larvae experienced no metabolic stress at pH 4.

Accelerated growth was demonstrated to result from indirect effects of low pH on the food resource. Dual labelling experiments revealed a more efficient utilization of leaf energy (^{14}C) and microbial energy (^{3}H) of pH 4-conditioned leaf litter. Higher nutritional quality of leaves at low pH was attributed to increased fungal abundance.

Accelerated shredder growth was offset by reduced survival, resulting in lower biomass production by larvae reared at pH 4 as compared to pH 6.

L'ACIDIFICATION D'EAU DOUCE: LES EFFETS DE BAS NIVEAUX DE pH SUR LA TRANSFORMATION DE L'ÉNERGIE DES DÉTRITUS PAR UNE PHRYGANE DÉCHIREUSE

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Les relations de cause à effet sous-tendant les communautés d'eau acidifiée demeurent pour la plus grande partie inconnues. Le but de ce travail était d'évaluer l'importance des changements induits par le pH dans l'étape détritivores-microbes-matière organique de la chaîne alimentaire aquatique en tant que mécanisme de changement dans la communauté.

Nous avons étudié l'hypothèse qui veut que l'acidité élevée réduit l'efficacité du transfert d'énergie par les invertébrés déchireux, et ceci directement par l'augmentation du coût de maintenance de l'organisme masticateur et indirectement en réduisant la valeur nutritive de la matière organique de la source de nourriture par l'inhibition de l'activité microbienne.

L'élevage de phryganes (Clistoronia magnifica - Limnephilidae) à bas pH eut pour effet, non pas de diminier, mais plutôt d'accélérer la croissance des larves. Les larves élevées à pH de 4.2-5.2, en plus de grossir plus vite que les larves élevées à pH de 5.8-6.4, produisirent aussi des nymphes et des adultes plus gros. Des études faites avec des détritus de feuilles marquées avec du ^{14}C n'indiquèrent aucun effet direct du pH sur la répartition du carbone ingéré, ce qui suggère que les larves ne subirent aucun stress métabolique à un pH de 4.

Il a été démontré que la croissance accélérée résulte des effets indirects du pH bas sur les ressources alimentaires. Des expériences de marquage double révélèrent une utilisation plus efficace de l'energie foliaire (^{14}C) et microbienne (^{3}H) pour des détritus foliaires conditionnés à pH 4. La plus haute valeur nutritive des feuilles à bas pH est attribuée à l'augmentation en abondance des champignons.

La croissance accélérée des insectes déchireurs fut contrebalancée par leur survie réduite, le tout résultant en une production de biomasse plus basse par les larves élevées à un pH de 4, comparée à celle des larves élevées à un pH de 6.

ENVIRONMENTAL POLLUTANTS AND FISH DISEASE/HEALTH

PAPERS AND ABSTRACTS

FISH NEOPLASMS: EPIZOOTIOLOGY, EXPERIMENTAL CARCINOGENESIS, RISKS AND BENEFITS

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Credible reports of neoplasms in reptiles, amphibians, fishes and bivalve mollusks began appearing in the literature approximately one century ago. Most notable of earlier cases was Ohlmacher's report in 1898 of lymphoma in northern pike (Esox lucius), a disease prevalent today in North America, Ireland and the Baltic Sea. Strong evidence supports a viral etiology, and if Ohlmacher had persisted in his studies, he, rather than F. Peyton Rous, might have won the Nobel Prize for discovering that viruses can cause some cancers.

The next half century added several hundred fish tumor case reports. James Johnstone of Liverpool, England, described 74 fish neoplasms in 23 papers from 1911-1927. Almost all were solitary cases, most were mesenchymal and none were in liver to suggest the presence of environmental carcinogens. Takahashi of Japan described nine new fish neoplasms in a lengthy review paper in 1929. Similarly, these were solitary cases none of which arose in liver.

Renal adenocarcinoma, once prevalent in leopard frogs (Rana pipiens) from Vermont to South Dakota, was first described in 1905. It contains nuclear inclusions and being transplantable, Baldwin Luke suggested it was caused by a virus. Electron microscopy in the 1950's revealed herpesvirus in the inclusions and provided the first evidence that a herpesvirus could be oncogenic. Today we suspect herpes viruses of causing Burkitt's lymphoma, nasopharyngeal carcinoma and Kaposi's sarcoma in humans. Liver cancer in rainbow trout (Salmo gairdneri), became panzootic with the widespread adoption of pelleted fish food. It was shown in the early 1960's to be caused by mold produced aflatoxins and this discovery of a whole new class of chemical carcinogens from fish studies has undoubtedly prevented many human cancers.

The discoveries of oncogenic herpesviruses in frogs and carcinogenic aflatoxins in fish encouraged the U.S. National Cancer Institute in the mid 1960's to create a Registry of Tumors in Lower Animals, in part to evaluate possible benefits from studies of neoplasms in the lower 99% of the animal kingdom. Computerized Registry specimen and reprint data bases show that after a century of low level study, fish neoplasms are known from all tissue systems and from 300 species. The causes of many types of fish tumors are unknown or unsubstantiated, but five fish tumors have evidence of being caused by viruses: lymphoma in northern pike and muskellunge (Esox masquinongy), swim bladder leiomyosarcoma in Atlantic salmon (Salmo salar), dermal fibroma in walleye (Stizostedion vitreum vitreum), and multiple epithelial neoplasms in chum salmon (Oncorhynchus keta) artificially infected with herpesvirus from non-tumorous cherry (masu) salmon (Oncorhynchus masou).

Many fish are sensitive to chemical carcinogens. Twenty-four fish species have developed neoplasms after experimental exposure to 30 mammalian carcinogens, including aflatoxins, PAH's, aromatic amines and nitrosamines. In nearly every study, hepatocellular and/or cholangiocellular cancers were seen, but other cancers also developed occasionally, including nephroblastoma, rhabdomyosarcoma, retinoblastoma, branchioblastoma, hemangioendothelioma, chromatoblastoma, squamous epidermal carcinoma and gastric adenocarcinoma. As the principal tissue for producing mixed function oxidases (MFO's) which metabolize the indirect carcinogens to the reactive genotoxic proximate carcinogenic moieties, it is logical that fish liver cancer is the most common type following most carcinogen exposures. With enzyme systems similar to mammals and with evidence for mammalian-type oncogenes in fish and invertebrates down the phylogenetic scale to sponges, it is not surprising that fish develop cancer after exposure to chemicals known to be carcinogenic in mammals.

Epizootic fish liver and skin cancers occur in heavily polluted habitats, indicating that carcinogens are entering the waterways. In addition to originating in liver, evidence for a chemical etiology includes epizootiology showing fish cancers clustered near concentrations of chemicals, and laboratory induction of fish and rodent tumors by extracts of sediment from polluted waterways where tumors are prevalent in the feral fish.

Experimental studies show that fish bioassays, especially those using embryos, are sound scientific methods to pre-screen chemicals for carcinogenicity with stunning practical advantages in cost and time. Sensitivity is excellent, interpretation is rarely complicated by spontaneous tumors, duration of chronic tests is 6-12 months versus two years for rodents and the cost is $20,000 versus at least $500,000 for rodents. Such bioassays could identify suspect carcinogens among new chemicals being considered for commercial use and among the 61,000 untested chemicals already in common usage. The suspect carcinogens could be funneled to rodent bioassays for corroboration.

Fish bioassays could also identify mixtures of air borne and water borne carcinogens entering the environment from point sources. Should extracts of outfall or smokestack effluents produce tumors in test fish, retrograde fish bioassays could locate the source. Complementarily, histopathological surveys of wild fish populations can indicate which aquatic ecosystems are contaminated by chemicals that cause cancer. This process may soon become more efficacious by the substitution of feral fish histopathology with standard laboratory bioassays of bile and liver extracts taken from the wild fish.

In conclusion, it now appears that among the major benefits of the study of cancer in fish will be use of fish bioassays and surveys to identify and eliminate point source environmental carcinogens.

NÉOPLASMES DE POISSONS: ÉPIZOOTIOLOGIE, CARCINOGÉNÈSE EXPÉRIMENTALE, RISQUES ET BÉNÉFICES

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Des rapports dignes de foi à propos de néoplasmes chez des reptiles, amphibiens, poissons et mollusques bivalves commencèrent à apparaître dans la littérature il y a environ un siècle. Le plus fameux de ces premiers cas fut le rapport par Ohlmacher, en 1898, à propos de lymphomes chez le grand brochet. Cette maladie est aujourd'hui répandue en Amérique du Nord, Irlande et dans la mer Baltique. L'évidence supporte fortement une étiologie virale, et si Ohlmacher avait persisté dans ses études, cela aurait pu être lui, plutôt que F. Peyton Rous, qui aurait gagné le prix Nobel pour la découverte que les virus peuvent être la cause de certains cancers.

Le demi-siècle suivant vit l'addition de plusieurs centaines de rapports à propos de tumeurs chez les poissons. James Johnston, de Liverpool, Angleterre, décrivit 74 néoplasmes de poissons dans 23 articles de 1911 à 1927. Ils étaient presque tous des cas particuliers, la plupart mésenchymateux et aucun dans le foie, ce qui aurait suggéré la présence de substances cancérigènes dans l'environnement. Takahashi, du Japon, décrivit neuf nouveaux néoplasmes de poissons dans une longue revue en 1929. Ces cas étaient aussi isolés et aucun d'eux ne provenait du foie.

L'adénocarcinome rénal, jadis répandu chez les grenouilles léopards (*Rana pipiens*) du Vermont jusqu'au Dakota du Sud, fut décrit pour la première fois en 1905. Il contient des inclusions nucléaires et le fait qu'il soit transplantable amena Baldwin Luke à suggérer qu'il soit causé par un virus. La microscopie électronique des années 1950 révéla un virus de la famille des herpétoviridés dans les inclusions et fournit la première évidence qu'un herpétoviridé puisse être oncogène. De nos jours nous pensons que les herpétoviridés puissent être la cause du lymphome de Burkitt, des carcinomes nasapharyngiens et du sarcome de kaposi chez les humains. Le cancer du foie chez la truite arc-en-ciel (*Salmo gairdneri*) devint universel avec l'adoption générale de la nourriture pour poissons en grains. On démontra au début des années '60 que ce cancer est causé par des moisissures produites par des aflatoxines et cette découverte a sans aucun doute prévenu plusieurs cancers humains.

Les découvertes d'herpétoviridés oncogènes chez les grenouilles et d'aflatoxines cancérigènes chez les poissons encouragèrent le "U.S. National Cancer Institute" à créer un Bureau d'Enregistrement des Tumeurs chez les Animaux Inférieurs dans le milieu des années '60, en partie dans le but d'évaluer les bénéfices possibles originant des études de néoplasmes parmi 99% des espèces qui consitutent la partie inférieure du règne animal. Les spécimens et les bases de données informatisées du Bureau d'Enregistrement montrent qu'après un siècle d'études sur les niveaux inférieurs, les néoplasmes de poissons sont répandus dans tous les tissus et chez 300 espèces. Bien que les causes de plusieurs types de tumeurs de poissons soient inconnues ou non confirmées, cinq tumeurs de poissons sont apparemment causées par des virus: les lymphomes chez le grand brochet et chez le maskinongé (*Esox masquinongy*), le léiomyosarcome de la vessie natatoire chez le saumon atlantique (*Salmo salar*), le fibrome dermique chez le doré (*Stizostedion vitreum vitreum*) et les néoplasmes épithéliaux multiples chez les saumons keta (*Oncorhynchus keta*) artificiellement infectés par des herpètoviridés provenant de saumons masou (*Oncorhynchus masou*) sans tumeur.

Plusieurs poissons sont sensibles aux substances cancérigènes chimiques. Vingt-quatre espèces de poissons ont développé des néoplasmes après avoir été exposés expérimentalement à 30 substances cancérigènes: mammifères incluant les aflatoxines, les amines aromatiques de HAP (Hydrocarbure Aromatique Polycyclique, ou PAH) et les nitrosamines. Dans presque chaque étude, des cancers hépatocellulaires et/ou cholangiocellulaires furent trouvés, mais d'autres cancers se développèrent aussi occasionnellement, dont des néphroblastomères, rhabdomyosardomes, rétinoblastomères, branchioblastomères, hémangio-endothéliomères, chromatoblastomères, carcinomes épidermiques squameux et des adénocarcinomes gastriques. Il semble logique que le cancer du foie chez le poisson soit le type de cancer le plus commun résultant d'expositions aux substances cancérigènes, étant donné que les tissus hépatiques sont les principaux producteurs d'oxydase à fonction mixte (OFM), qui métabolise des substances cancérigènes indirectes jusqu'aux moitiés cancérigènes génotoxiques proximales réactives. Etant donné qu'ils possèdent des systèmes enzymatiques similaires à ceux des mammifères et qu'il y a évidence d'oncogènes de type mammifère chez les poissons, les invertébrés et en descendant l'arbre phylogénétique jusqu'aux éponges, il n'est pas surprenant que les poissons développent des cancers après avoir été exposés à des produits chimiques reconnus comme étant cancérigènes pour les mammifères.

Les cancers épizootiques de la peau et du foie chez les poissons surviennent dans les habitats hautement pollués, ce qui indique que les substances cancérigènes sont en train d'envahir les cours d'eau. L'évidence pour une étiologie chimique, en plus d'originer du foie, inclut une épizootiologie retraçeant des cancers chez les poissons qui sont groupés près de concentrations de produits chimiques et des inductions en laboratoire de tumeurs chez des poissons et chez des rongeurs par des extraits de sédiments provenant de cours d'eau pollués où ces tumeurs sont répandues chez les poissons qui les habitent.

Les études expérimentales démontrent que les essais biologiques avec poissons, surtout ceux utilisant des embryons, sont des méthodes scientifiques trés valables pour dépister les produits chimiques pouvant causer le cancer, et ceci avec des avantages pratiques énormes en temps et en coût. Leur sensitivité est excellente, leur interprétation est rarement compliquée par des tumeurs spontanées, la durée des tests chroniques est de 6 à 12 mois comparé à deux ans pour les rongeurs et leur coût est de $20,000, comparé à un minimum de $500,000 pour les essais avec rongeurs. De tels essais biologiques pourraient identifier des substances qu'on suspecte être cancérigènes parmi les nouveaux produits chimiques étant considérés pour utilisation commerciale et parmi les 61,000 produits chimiques non testés qui sont déjà d'usage commun. Les substances cancérigènes suspectes pourraient être canalisées à travers les essais biologiques avec rongeurs pour collaboration.

Les essais biologiques avec poissons pourraient aussi identifier des mélanges de substances cancérigènes aériennes et aqueuses entrant dans l'environnement à partir de diverses sources. Si les extraits de retombées ou d'effluents des cheminées produisent des tumeurs chez les poissons testés, des essais biologiques rétrogradés avec poissons pourraient en localiser la source. D'une façon complémentaire, des inspections de populations sauvages de poissons peuvent indiquer quels écosystèmes aquatiques sont contaminés par des produits chimiques causant le cancer. L'efficacité de ce procédé va peut-être bientôt être accrue par la substitution de l'histopathologie par des essais biologiques standard en laboratoire sur des extraits de bile et de foie pris à partir de poissons sauvages.

En conclusion, il semble que l'utilisation d'essais biologiques avec poissons et les inspections pour identifier et éliminer les sources de substances cancérigènes environnementales soient parmi les bénéfices majeurs de l'étude du cancer chez les poissons.

CONCEPTUAL BASIS FOR THE BIOCHEMICAL SURVEILLANCE OF CONTAMINANT EFFECTS ON FISH

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Laboratory toxicity tests are used to predict the impacts of toxic chemicals on aquatic ecosystems. These predictions are expressed as water quality objectives, or the levels of chemicals 'safe' for aquatic biota. Water quality is managed through sufficient waste treatment that objectives are met during routine chemical monitoring; protection of the aquatic ecosystem is then assumed.

This approach, while productive, cannot prevent the 'unplanned event' such as unexpected chemical interactions or the unexpected occurence of toxic chemicals (e.g. mirex in Lake Ontario). Hence, biological surveillance of ecosystem health has often been recommended to detect and correct these problems. However, significant adverse effects on aquatic biota cannot be prevented if those effects form the basis for surveillance. Recovery of community structure or loss of chemicals from fish may take years if the system is large or the chemicals persistent.

All chemical toxicity to ecosystems, communities and populations starts with a chemical event in an individual. Hence, measuring that chemical event provides a better tool for effects surveillance than responses of populations or ecosystems. It will occur before these more serious responses, at lower levels of exposure, and will be specific to one chemical or group of chemicals. The effects on an individual may often be reversible if the exposure ceases.

The main difficulty with this approach is a failure by managers to understand, and by scientists to demonstrate, the link between biochemical responses and important ecological effects. The link can be established conceptually by an 'ecosystem stress-response matrix' (Dixon et al., 1985).

The first chemical reactions arising from chemical exposure are equivalent to the primary stress response of Selye's 'General Adaptation Syndrome' (Wedemeyer et al., 1948). More severe exposure leads to compensation by the individual (e.g. detoxification) which may have energetic costs. This secondary response may cause some primary responses of populations such as reduced size at age. Further exposure of individuals may lead to a tertiary response or crisis; i.e. a failure to compensate. The result is significant chronic effects on the rates of growth, reproduction or mortality of populations, and compensatory reactions such as increased fecundity in response to increased rates of adult mortality.

The secondary response of populations can induce primary changes in communities (e.g. in the balance between predator and prey populations) since interactions among species will alter. More severe exposures that cause the rapid mortality of individuals or failure to reproduce will induce a population crisis and compensation at the community levels, as species dominance and composition changes. These shifts induce primary changes in ecosystems shown by changes in energy flow, biomass spectra, etc.

While these cause-effect relationships are highly idealized, they provide a model for studies to show how the responses of individuals to chemicals may generate ecological effects. Such studies, and the development of specific biochemical diagnostic tools, are urgently needed to support biological surveillance that forecasts ecosystem effects.

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BASE CONCEPTUELLE DE LA SURVEILLANCE BIOCHIMIQUE DES EFFETS DES CONTAMINANTS SUR LES POISSONS

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Les tests de toxicité menés en laboratoire servent à prédire l'impact qu'ont les substances chimiques toxiques sur les écosystèmes aquatiques. Ces prédictions sont exprimées en termes d'objectifs pour la qualité de l'eau, ou en termes de niveaux 'sécuritaires' de produits chimiques dans le milieu aquatique. La qualité de l'eau est régie à travers des traitements d'eaux usées suffisant pour atteindre les objectifs lors d'examens routiniers des produits chimiques; la protection de l'écosystème aquatique est par après assumée.

Cette approche ne peut cependant prévenir les "événements imprévus" tels que des interactions chimiques inattendues ou l'apparition soudaine de produits chimiques toxiques (e.g. le mirex dans le lac Ontario). Par conséquent, la surveillance biologique de la santé des écosystèmes a souvent été prescrite pour détecter et corriger ces problèmes. Cependant, les effets adverses significatifs sur le milieu aquatique ne peuvent être prévenus si ces mêmes effets forment la base de la surveillance. La réhabilitation de la structure d'une communauté ou la perte de produits chimiques assimilés par des poissons peuvent prendre des années si le système est grand ou si les produits chimiques sont persistants.

Toute toxicité chimique, qu'elle soit dans un écosystème, dans une communauté ou dans une population, commence par un événement à base chimique dans un individu. Donc, la mesure de cet événement chimique est un meilleur outil pour la surveillance des effets que les réponses de populations ou d'écosystèmes. L'événement au niveau individuel arrivera avant les réponses plus sérieuses, à de plus bas niveaux d'exposition, et sera spécifique à un produit chimique ou à un groupe de produits chimiques. Les effets au niveau individuel sont souvent réversibles si l'exposition cesse.

La principale difficulté avec cette approche réside dans un manque de compréhension de la part des administrateurs et dans un manque de preuves de la part des chercheurs qu'il existe un lien entre les réponses biochimiques et les importants effets écologiques. Ce lien peut être établi conceptuellement, en tant que 'matrice de stress et réponse au niveau de l'écosystème' (Dixon et al., 1985).

Les premières réactions chimiques résultant d'une exposition à des produits chimiques sont équivalentes à la réponse primaire au stress dans le 'Syndrome d'Adaptation Générale' de Selye (Wedemeyer et al., 1984). Une exposition plus intense mène à la compensation par l'individu (e.g. désintoxification), ce qui peut encourir des coûts en énergie. Cette réponse secondaire peut, dans une population, résulter en des réponses primaires telles que des réductions dans la grosseur et dans la survie. Une exposition encore plus sévère peut mener à une réponse tertiaire ou crise, i.e. à un arrêt de compensation. Les résultats en sont des effets chroniques significatifs sur le taux de croissance, de reproduction ou de mortalité des populations, et des réactions compensatrices telles une fécondité accrue en réponse à des taux accrus de mortalité chez les adultes.

La réponse secondaire des populations peut induire des changements primaires dans les communautés (e.g. la balance entre populations de prédateurs et de proies) vu que les les interactions interspécifiques vont changer. Des expositions de plus en plus sévères causant une mortalité rapide chez les individus ou une absence de reproduction, vont induire une crise au niveau de la population et une compensation au niveau de la communauté, à mesure que la dominance et la composition des epèces changent. Ces changements induisent des changements primaires dans les écosystèmes à travers des changements dans la circulation de l'énergie, le registre de la biomasse, etc.

Bien que ces relations de cause à effet soient hautement idéalisées, elles fournissent un modèle pour les etudes qui ont à determiner comment les reponses individuelles aux produits chimiques peuvent genérer des effets ecologiques. Ces etudes, de meme que le developpement d'outils pour le diagnostique biochimique, sont pressantes si l'on veut supporter une surveillance biologique capable de predire les consequences au niveau de l'ecosysteme.

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STUDIES OF BROOK TROUT, SALVELINUS FONTINALIS, DENSITY, GROWTH AND MOVEMENT IN ICEWATER CREEK, ALGOMA DISTRICT, ONTARIO

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The Forest Pest Management Institute has initiated an assessment of brook trout, Salvelinus fontinalis, populations as part of a continuing research project at Icewater Creek, a small coldwater tributary of the Goulais River within the Lake Superior drainage system. The assessment has been designed to provide baseline data against which to evaluate secondary effects on brook trout of a forest pesticide induced disturbance of benthos within a treated section of stream. The stream morphology of upper Icewater Creek allows for concomitant study of trout populations in both treated and untreated sections. Aquatic invertebrate density and distribution sampling has also been part of the baseline data collection, but is not covered in this poster.

Two years of pretreatment data have been collected to date from brook trout populations in the two major headwater tributaries of Icewater Creek. Sampling protocol has focused on determining seasonal changes in trout density and growth in designated sampling sections, as well as fish movement and distribution patterns within the entire headwater area. The results of these data collections are presented, and their implications to the forthcoming assessment of brook trout responses to pesticide application in Icewater Creek are discussed.

ÉTUDES SUR LA DENSITÉ, LA CROISSANCE ET LE MOUVEMENT DE TRUITES MOUCHETÉES (SALVELINUS FONTINALIS) DANS ICEWATER CREEK, DISTRICT D'ALGOMA, ONTARIO

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Le "Forest Pest Management Institute" a initié une évaluation de l'état des populations de truites mouchetées, Salvelinus fontinalis, faisant partie d'un projet de recherche à Icewater Creek, petit affluent de la rivière Goulais qui fait parti du bassin de drainage du lac Supérieur. L'évaluation fut conçue afin de fournir des données de base sur lesquelles on puisse s'appuyer pour évaluer les effets secondaires, sur les truites mouchetées, des perturbations de benthos induites par un pesticide utilisé dans la forêt le long d'une section de ruisseau. La morphologie de la partie amont de Icewater Creek permet des études concomitantes de populations de truites dans des sections traitées et non-traitées. Un échantillonnage des invertébrés aquatiques et de leur distribution fait aussi partie de la collection des données de base, mais n'est pas inclus dans ce poster.

Jusqu'à date, deux années de données avant traitement ont été accumulées sur les populations de truites mouchetées dans les deux affluents majeurs de Icewater Creek. Le protocole d'échantillonnage a été concentré sur la détermination des changements saisonniers dans la densité des truites et dans leur croissance dans des sections particulières d'échantillonnage, ainsi que sur les mouvements et les types de distribution des poissons dans tout le bassin. Nous présentons les résultats de ces collections de données et nous discutons leurs implications dans l'évaluation future des réponses des truites mouchetées aux applications de pesticides dans Icewater Creek.

EXTENDED ABSRACT

The Forest Pest Management Institute has initiated an assessment of brook trout, Salvelinus fontinalis, populations as part of a continuing research project at Icewater Creek, a small coldwater tributary of the Goulais River within the Lake Superior drainage system. The assessment has been designed to provide baseline data against which to evaluate secondary effects on brook trout from forest pesticide induced disturbances of benthos within treated sections of stream.

Density estimates of brook trout in each of the study sections, calculated with a maximum weighted likelihood estimation model, varied throughout the sampling season (May to October), with spatial differences more apparent than seasonal trends. Capture fractions determined for each sample date indicated that percent recaptures ranged from 10 to 76, with an overall recapture rate of approximately 30%. Trout in the current year cohort appeared in the fish collections by late May, increased in percent composition of the total catch throughout the season and, represented 50 to 85% of the trout present in the study areas by October. Movement of brook trout in the upper watershed was apparently limited throughout the sampling season with 56 to 84% of marked (hot-branded) individuals recaptured within 50 m of their original capture site, and less than 12% recaptured at distances greater than 200 m. The recapture of marked fish allowed for direct measurement of growth of individual brook trout from different times of the year. Fish recaptured within specific time segments were pooled and instantaneous rates of growth were calculated for different seasons. Length-weight regressions were determined and plotted for trout collected at the end of their first year of growth to facilitate annual comparison of growth of trout in the current year cohort. Annual trout production estimates for the sampling areas ranged from 5.13 to 18.97 kg/ha and mean annual standing stocks ranged from 3.41 to 12.79 kg/ha.

MATACIL MAY ENHANCE SUSCEPTIBILITY OF FISH CELLS TO VIRAL INFECTION

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Matacil, an aminocarb insecticide used in control of spruce budworm, has significantly reduced cellular multiplication and caused serious cytotoxic changes in our previous experiments. We postulated that this insecticide might have an effect on cellular susceptibility to viral infection. Monolayer cultures of fathead minnow (FHM) cells were treated with various concentrations of commercially available matacil for one hour before they were inoculated with dilutions of LT-1 virus. The inoculated cultures were covered with a liquid overlay for seven days. The cultures were fixed and stained with Paragon blue containing 10% formalin. The number and size of plaques that developed in the monolayers, treated with the different concentrations of matacil, were evaluated. The results suggest that matacil may enhance viral infection of fish cells *in vitro*.

LE MATACIL PEUT ACCROÎTRE LA SENSIBILITÉ DES CELLULES DE POISSONS À L'INFECTION VIRALE

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Le matacil, un insecticide carbaminé utilisé dans le contrôle de la tordeuse du bourgeon d'épinette, a réduit significativement la multiplication cellulaire et a causé de sérieux changements cytotoxiques lors d'expériences précédentes. Nous avons postulé que cet insecticide puisse avoir un effet sur la susceptibilité cellulaire à l'infection virale. Des cultures monomoléculaires de cellules de vairon imbécile (VI) furent traitées pour une heure avec diverses concentrations de matacil commercialement disponible, avant d'être inoculées avec des dilutions de virus LT-1. Les cultures inoculées furent couvertes d'un revêtement liquide pour 7 jours. Les cultures furent fixées et teintes avec du bleu de Paragon contenant du formol à 10%. On évalua le nombre et la grosseur des plaques qui se développèrent dans les strates traitées avec différentes concentrations de matacil. Les résultats suggèrent que le matacil augmente l'infection virale des cellules de poissons *in vitro*.

INTRODUCTION

During the past decade the organophosphate and carbamate insecticides have been used extensively for controlling spruce budworm since they degenerate in a short period after application and appear to have a relatively low toxicity to fish and mammals. Their mode of action and long term sub-lethal effects have never been fully studied. In our previous work Matacil, a carbamate insecticide, caused serious cytological changes and inhibited cellular multiplication (Li et al., 1981). The present report describes fish cell monolayer cultures exposed to various concentrations of commercial Matacil. Preliminary results are presented.

MATERIALS AND METHODS

Matacil solution

Commercial Matacil stock solution was supplied by P. Wells of Environment Canada. The Matacil was suspended in Hanks' balanced salt solution (BSS) to desired concentration with a Vortex* mixer.

Tissue cultures

A fish cell culture, Fathead minnow, was maintained in minimal essential medium (MEM) supplemented with 10% fetal bovine serum. After sub-cultivation in 25 cm^2 tissue culture flasks the seven-day old monolayer cultures were exposed to various concentrations of the Matacil solution prior to infection with an amphibian virus (LT-1). The Matacil solutions were added to the cultures for two hours at 18°C. At the end of the two-hour period the Matacil solutions were decanted and the cell monolayers were washed once with BSS before adding the liquid overlay growth medium containing 0.1 ml of virus preparation (1500 pfu).

Virus stock solution

The amphibian virus (LT-1) was obtained from Dr. Fred Clark of the Wistar Institute in Philadelphia. The virus was propagated in FHM cells and stored at -40°C. Before inoculation the stock virus solution was diluted 1:100 (approximately 1500 pfu/0.1 ml) in BSS.

Plaque assay

At the end of a seven-day incubation period the monolayers were fixed and stained with Paragon** solution containing 10% formalin (Wheeler et al., 1968). The size and number of the plaques formed by the virus on the monolayers were evaluated as parameters of this experiment.

RESULTS AND DISCUSSION

Figure 1 shows a monolayer of FHM cells, with no previous exposure to

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*Scientific Industries Inc., Springfield 3, Massachusetts.

**Paragon C. & C. Co., Inc., 190 Willow Avenue, Bronx, N.Y. 10454.

Matacil, infected with LT-1 virus. Figure 2 is a monolayer culture exposed to 5 ppm Matacil prior to inoculation with the virus. The cells, once exposed to the insecticide, appeared to be more easily lysed by the virus.

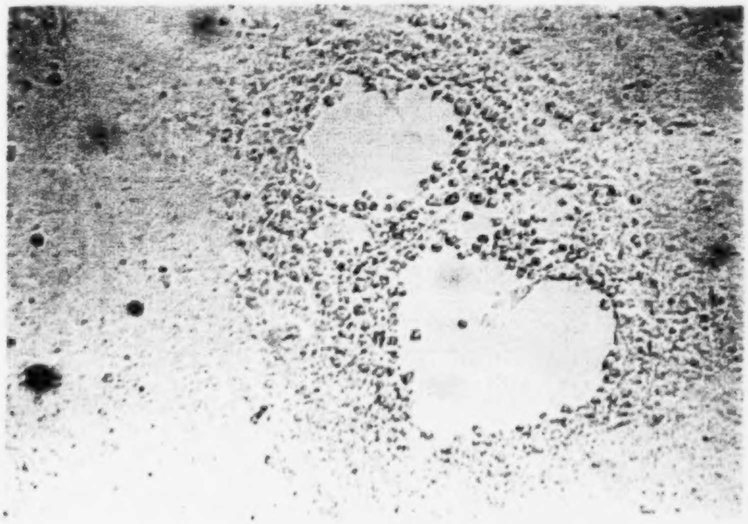

Figure 1. A control monolayer of FHM cells infected with LT-1 virus. Mag. 700X.

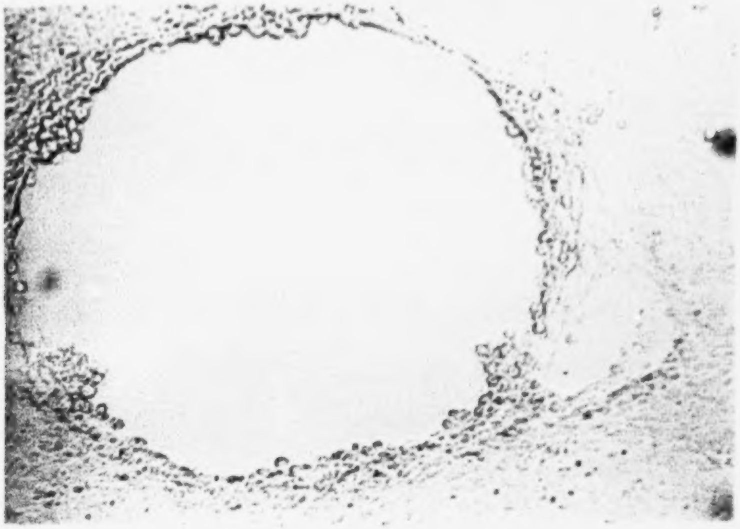

Figure 2. A monolayer of FHM cells infected with LT-1 virus after previous exposure to 5 ppm Matacil. Mag. 700X.

For seven days the exposed and unexposed cells were inoculated with the same amount of liquid overlay virus solution (1500 pfu). One of the typical results is shown in Table 1.

Table 1. Effect of matacil (concentrate) on susceptibility of FHM cells to amphibian virus (LT-1).

Matacil Concentration (PPM)	Plaque Size (mm)		Plaques per Flask
	Mean	Range	
0	0.4	0.1 - 0.6	75
5	0.5	0.1 - 0.8	106
10	0.62	0.1 - 1.0	132
20	0.69	0.1 - 1.2	127

Each flask was inoculated with 1500 pfu's of virus suspension. The cultures were incubated at 18°C for seven days.

Our preliminary results indicate that at the end of seven days the exposed monolayers had more numerous and larger plaques than those found in the control monolayers. This suggests that the cells became more susceptible to the viral infection. Whether the observed effect was due to the insecticide or to the solvents used in the commercial Matacil solution was not evaluated. Some degree of variation did occur from trial to trial.

Pesticides and detergents are among the most destructive types of pollutants to which populations of fish and shellfish are exposed (Sprague, 1967). According to most favored etiological hypotheses, Reye's Syndrome stems from a virus-host interaction, possibly modified by some exogenous agent or agents (Lancet, 1982), and it has also been suggested that certain insecticides may be related to this syndrome (Crocker et al., 1974). Our preliminary results show that commercial Matacil stock solution enhances viral susceptibility of fish cells. More work is needed in order to make statistical analysis possible.

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^{14}C-OCTACHLORODIBENZO-P-DIOXIN: FATE IN ARTIFICIAL OUTDOOR PONDS

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Artificial outdoor ponds (5200 L) were treated with ^{14}C-octachloro-dibenzo-p-dioxin (O_8CDD) to yield initial concentrations of 0, 340, and 680 ng/L. The O_8CDD was dissolved in tetrahydrofuran, coated onto sodium chloride crystals and applied to the surface of the ponds. During the 51-day experiment begun August 17, 1983, the concentration of ^{14}C-O_8CDD was determined in water, sediment, vegetation, and fish from the ponds. In addition, volatile loss of ^{14}C-O_8CDD was also followed; it reached a maximum within the the first day post-treatment. Concentrations in the water were, immediately after treatment, 32 ng/L in the 340 ng/L pond and 89 ng/L in the 680 ng/L pond; levels decreased steadily and at two hours post-treatment were 17 ng/L and 19 ng/L respectively. Levels in sediment, fish and vegetation were also quantified.

LE SORT DE LA ^{14}C-OCTACHLORODIBENZO-P-DIOXINE DANS DES ÉTANGS ARTIFICIELS

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Des étangs artificiels d'extérieur (5200 L) furent traités avec de la ^{14}C-octachlorodibenzo-p-dioxine (O_8CDD) à des concentrations initiales de 0, 340 et 680 ng/L. La O_8CDD fut dissoute dans du têtrahydrofuran, on enroba cette mixture de cristaux de chlore et on appliqua le tout à la surface des étangs. Durant les 51 jours de l'expérience, commencée le 17 août 1983, la concentration de ^{14}C-O_8CDD fut déterminée dans l'eau, les sédiments, la végétation et les poissons des étangs. De plus, on traça aussi les pertes de ^{14}C-O_8CDD par évaporation; ces pertes atteignirent un maximum le premier jour après traitement. Immédiatement après le traitement, les concentrations dans l'eau étaient de 32 ng/L dans l'étang ayant 340 ng/L et de 89 ng/L dans l'étang ayant 680 ng/L; les niveaux diminuèrent rapidement, et deux heures après l'initiation du traitement, ils n'étaient déjà plus que de 17 ng/L et 19 ng/L respectivement. Les niveaux présents dans les sédiments, les poissons et la végétation furent aussi quantifiés.

INTRODUCTION

O_8CDD may enter the environment from many different sources, but its presence as a contaminant of the fungicide and wood preservative pentachlorophenol, at levels sometimes as high as 1380ppm (Goldstein et al., 1977) represents an important mode of entry. The distribution and persistence of O_8CDD added to an aquatic ecosystem will depend upon the physicochemical properties of the compound and the limnological characteristics of the ecosystem. The water solubility of O_8CDD is extremely low (3.85×10^{-10} g/L, Webster et al., 19[illegible] and it is strongly sorbed onto organic matter and surfaces of various kinds. In the absence of data from the field, it is very difficult to predict, with any confidence, the fate and biological effects of such a chemical in natural waters. A pond study, similar to the work of Corbet et al., (1983) was therefore carried out to obtain data on dispersion of O_8CDD in the field.

Description of the test system

The experiment was carried out in small experimental ponds (4 m x 5 m x 0.9 m deep) located at the Glenlea Research Station, 20 km south of Winnipeg. Ponds were lined with 10 mil polyethylene, covered with 5 cm sand, 10 cm excavated soil and on the sides, with 2 layers of 5 cm thick clay-based sod. Finished ponds were then filled with water (final volume of 5 m^3) and left to acclimate for one year. Fathead minnows (100) were added to each pond one month in advance of treatment.

Application of ^{14}C-O_8CDD

On August 17, 1983, sodium chloride crystals coated with ^{14}C-O_8CDD were sprinkled onto the water and released their dioxin content into the water body. Pond 2 was treated with ^{14}C-O_8CDD to yield 340 ng/L, pond 5, 680 ng/L, and pond 4 was left as an untreated pond.

Residue sampling and analysis

Air was sampled by means of polyurethane foam plugs from 3 different heights above the water surface (5, 25 and 50 cm) up to 36 hours post-treatment, at 2 h intervals. Water was sampled sub-surface and extraction with dichloromethane was begun in the field. Sediment was sampled regularly and wet samples soxhlet extracted with tetrahydrofuran (THF) for 12 h. Fish were also sampled regularly and ball-mill extracted with THF. Vegetation was also sampled at regular intervals and samples held at -40°C while awaiting analysis. Foam plugs were soxhlet extracted with hexane for 2 h. All final extracts were analyzed by Liquid Scintillation Counting (LSC).

RESULTS AND DISCUSSION

Volatilization was important within the first day in both ponds (see Table 1) but factors such as wind speed and turbulence may have prevented us from observing a clear trend in the concentration variations. An evident trend was observed for the concentration variations of ^{14}C-O_8CDD in the water. Concentrations decreased rapidly within the first 8 days post-treatment, going from 32 ng/L to 1.5 ng/L in pond 2 and from 89 ng/L to 4.1 ng/L in pond 5 (see Figure

Table 1. Amount of $^{14}C\text{-}O_8$ CDD trapped over a 12 hour period (pg/L).

	Applied dose rate (ng/L)					
	340 ng/L			680 ng/L		
Height above water surface (cm)	5	25	50	5	25	50
Time (hrs)						
12	1.60	15.31	1.15	1.70	0.86	0.72
24	0.16	3.04	1.71	0.56	0.82	1.72
36	20.72	0.67	1.37	3.78	0.73	1.15

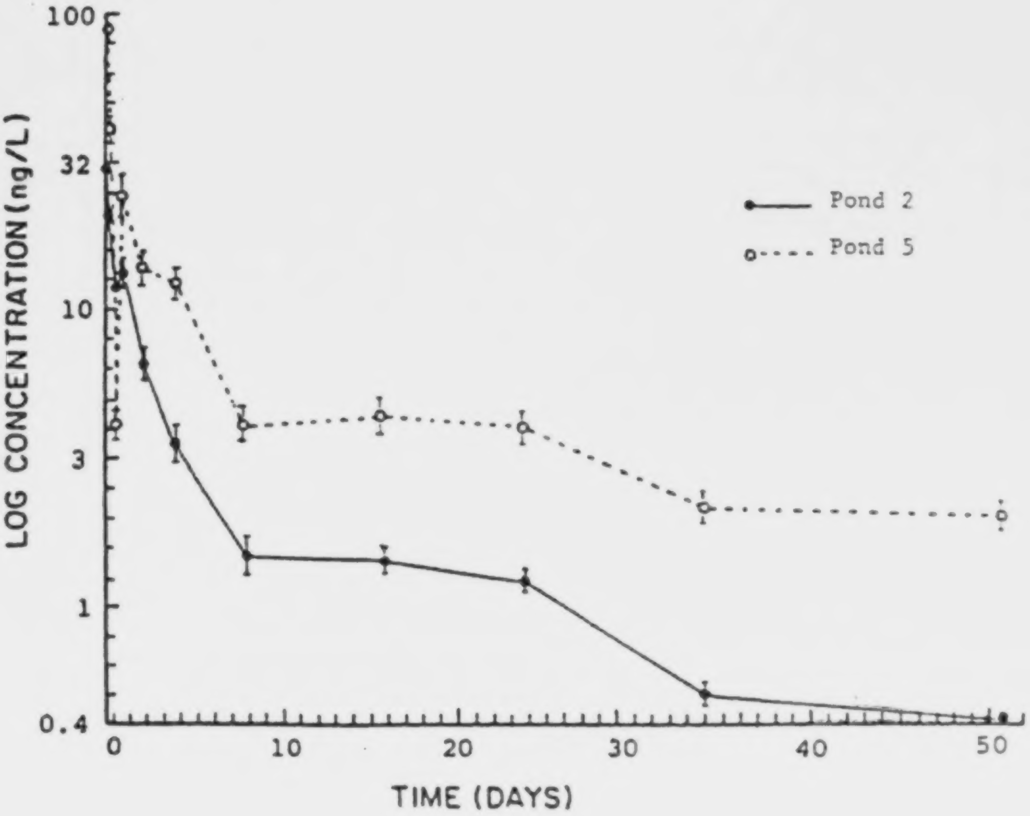

Figure 1. Presence of O_8CDD in pond water.

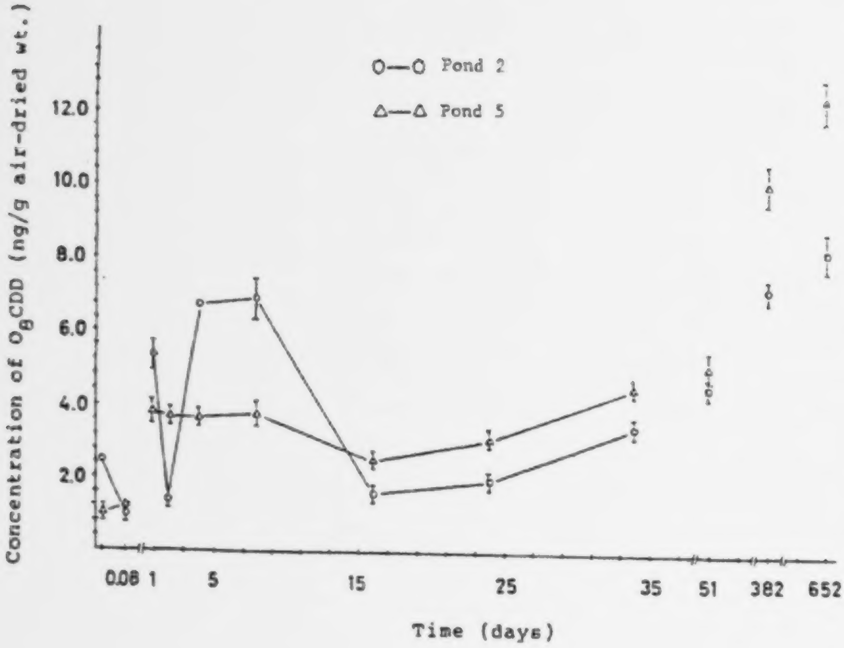

Figure 2. Sediment levels of O_8CDD.

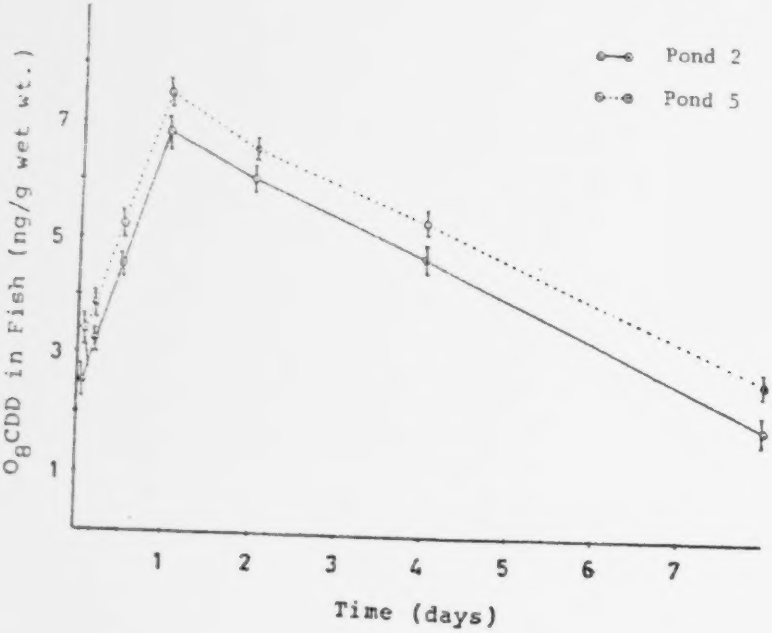

Figure 3. Presence of O_8CDD in fish.

1). From day 8 on, concentrations decreased very slowly to reach 2.0 ng/L in pond 5 on day 51 and at that time, no $^{14}C-O_8CDD$ was detectable in pond 2. Sediments appeared to be an important sink for the chemical since on day 382 of the experiment, pond 2 had accumulated 7.49 ng/g air-dried weight and pond 5, 10.1 ng/g (see Figure 2). In the early stages of the experiment, the fish bioconcentrated the dioxin very rapidly (see Figure 3). By day 1, concentrations had reached a maximum and were 6.86 ng/g wet weight in pond 2 and 7.51 ng/g wet weight in pond 5. From day 1 until day 8 concentrations decreased very rapidly. On day 8, BCF were 1260 in pond 2 and 660 in pond 5. In common with other persistent chemicals, O_8CDD is relatively stable under variable conditions of temperature, humidity and solar radiation. Consideration of its physico-chemical properities, in relation to its distribution in pond water, leads to the conclusion that adsorption to various media and organisms and volatilization were most probably the predominant processes involved in its dispersion and loss in the pond experiment.

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ACUTE AND DELAYED EFFECTS OF THE MUTAGEN ETHYL METHANESULPHONATE ON *BRACHYDANIO RERIO*

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Zebrafish (*Brachydanio rerio*) embryos and fry were exposed to variable concentrations of ethyl methanesulphonate (EMS). Observations of acute effects included a 96 hour LC_{50} of 700 mg/L and an embryo-larval MATC of 62-125 mg/L. Acute genotoxic damage visible at anaphase (AA's) due to 1 to 1000 mg/L EMS (24 hours) appeared to be responsible for micronuclei and cell death. Delayed mortality due to EMS began 9 days after 24 hours exposure of blastulas (100 and 1000 mg/L), and 16 days after exposure of fry to four 24 hour treatments (1000 mg/L). Abnormal colouration in adult zebrafish exposed 6 months previously as fry may be due to teratogenic or mutagenic changes in pigment cells. Large numbers of dead cells observed in the epidermis of these adults may represent heritable lethal mutations. Similar observations were not made for zebrafish exposed as blastulas. Anaphase aberrations in embryos lead to cell death, and micronuclei. Cell death itself may also contribute to micronuclei formation, as micronuclei were induced by a non-mutagen causing cell death, which resulted in the observed teratogenesis. Anaphase analysis reveals a wider variety of chromosomal and division apparatus aberrations than metaphase analysis, however it is more difficult to interpret. Observable defects may arise by breakage, induced stickiness, or a combination of both, while breakage events are the only defects visible at metaphase. Younger embryos were more sensitive for AA analysis, while embryonic erythropoietic tissue was more sensitive than yolk-sac cells for micronuclei analysis. The failure of the potent mutagen EMS to induce neoplasia may reflect a lack of promotion as numerous mutations (initiations) were induced.

LES EFFETS AIGUS ET RETARDÉS DE L'AGENT MUTAGÈNE ÉTHYLE MÉTHANESULPHONATE SUR BRACHYDANIO RERIO

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Des embryons et des alevins de poissons zèbre (Brachydanio rerio) furent exposés à des concentrations variées d'éthyle méthanesulphonate (EMS). Les observations des effets aigus comprenaient un CL_{50} de 96 heures de 700 mg/L et un MATC d'embryons et larves de 62-125 mg/L. Les dommages génotoxiques aigus visibles à l'anaphase (AA's) dûs à 1 à 1000 mg/L d'EMS (24 heures) apparurent être responsables pour les micronucléi et pour la mort des cellules. Une mortalité retardée due au EMS commença 9 jours après 24 heures d'exposition de la blastula (100 à 1000 mg/L), et 16 jours après l'exposition des alevins à quatre traitements de 24 heures (1000 mg/L). La coloration anormale des poissons zèbre adultes exposés 6 mois auparavant en tant qu'alevins peut être due à des changements tératogènes ou mutagènes dans les cellules de pigment. Le grand nombre de cellules mortes observées dans l'épiderme de ces adultes peut représenter des mutations héréditaires et létales. Des observations similaires ne furent pas conduites pour les poissons zèbre exposés à l'état de blastula. Des aberrations à l'anaphase, dans les embryons, menèrent à la mort des cellules et aux micronucléi. La mort des cellules en tant que telle peut aussi contribuer à la formation des micronucléi, étant donné qu'ils sont induits par un agent non mutagène causant la mort des cellules, ce qui résulte en la tératogénèse observée. L'analyse de l'anaphase révèle une plus grande variété d'aberrations chromosomales et d'appareils de division que l'analyse de la métaphase. Elle est cependant plus difficile à interpréter. Les défauts observés peuvent survenir par la rupture, l'induction de caractère gluant, ou d'une combinaison des deux, alors que les événements de rupture sont les seuls défauts visibles à la métaphase. Les embryons les plus jeunes sont plus sensibles aux analyses AA, alors que le tissu embryonnaire érythropoiétique est plus sensible que les cellules du sac vitellin pour les analyses de micronucléi. L'échec du puissant agent mutagène EMS à induire la néoplasie peut refléter un manque de promotion, étant donné que de nombreuses mutations (initiations) furent induites.

EXTENDED ABSTRACT

Exposure of embryo and fry stages of zebrafish (Brachydanio rerio) to solutions of the mutagen ethyl methanesulphonate (EMS) and a negative control compound (methyl isobutylketone; MIK) resulted in a variety of measureable effects. Acute lethality testing of both compounds, assessed with a 96 h static-renewal test, led to an LC_{50} of 700 mg/L for both compounds, with zebrafish fry as test organisms. Embryo-larval testing with EMS (8 days) led to a Maximum Acceptable Toxicant Concentration (MATC) encompassing the range of 62-125 mg/L, giving an application factor of approximately 0.1-0.2. The MATC for MIK, based on a four day test was 10-100 mg/L, the application factors thus being somewhat lower than for EMS (0.014-0.14). For both test chemicals the most sensitive response was teratogenesis.

Two exposure regimes were utilized in an attempt to induce neoplasia. Embryos were exposed to the test solution (EMS only) for 24 hours immediately after hatching, while juvenile fish were exposed for 4 24-h periods to the same concentrations. After exposure, the fish were maintained in dechlorinated water for a 6 month observation period. Mortalities were observed 9 days after embryo exposures, and 16 days after fry exposure. This delayed mortality resulted in 72% and 10% mortality in 1000 and 100 mg/L groups exposed as embryos, and 83% mortality in fry exposed to 1000 mg/L. The cause of these mortalities is unknown, though teratogenesis may be a possibility in fish exposed as embryos. Observations of surviving fish exposed as fry showed abnormal colouration patterns, the severity of which appeared to be related to the original exposure concentration, resulting in a wavy, broken pattern of yellow lines replacing the normal straight lines. Histological examination of the epidermis from these organisms revealed large numbers of pyknotic and karyorrhectic cells (Malpighian). Due to the extended interval between toxicant exposure and histopathological examination (130 days), it is hypothesized that the observed cell death was due to apoptosis, the result of induced mutations. Induced mutations and/or teratogenesis may have been responsible for the disorderly arrangement of the pigment cells in these fish. Similar observations were not made for the fish exposed as embryos. The exposure of embryos for 24 hours to 1000 mg/L induced grossly visible teratogenesis in 87% of the survivors on day 7, prior to the onset of delayed mortality. Histopathological examinations 5 months after exposure, after the onset of maturity, revealed no neoplasia in embryos exposed to 1000 mg/L as embryos; 50% of the surviving fish were examined. Given that it has been shown that neoplasia is inducible in a similar manner with aflatoxin B_1 and 7-12-dimethyl-a-anthracene (Hendricks, 1982; Schultz and Schultz, 1982) and is observable prior to maturation, it may be that an absence of a promotion stage reduced the probability of neoplasia developing. Alternatively, it may have been that severely affected fish died during the period of delayed mortality.

Analysis of chromosome patterns was undertaken to determine the relative sensitivity of zebrafish embryos for detecting genotoxicants. Exposure of embryos to EMS resulted in time and concentration dependent increases in chromosome breakage. This was visible at anaphase in embryo squashes, and proved to be an approach as sensitive as published results with adult fish. Six and 12 hour exposure to 100 mg/L EMS resulted in a significant increase in a variety of chromosome defects, while exposure for 24 hours to 1 mg/L elicited a significant increase. A 6 h delay in the initiation of exposure (embryos

exposed from 8 hours of age to 32 hours) resulted in a significant loss in apparent sensitivity. It is unknown whether exposure for the first 32 hours would be more sensitive than only 24 hours.

The advantage of a 32 h exposure is the initiation of erythrocyte formation, in embryonic hemopoietic tissue, which is primarily hepatic. Analysis revealed that 24 hour exposure of yolk sac cells was much less sensitive than the same exposure period culminating in the analysis of erythropoietic tissue. Chromosome breakage was revealed as micronuclei in both erythrocytes and yolk-sac cells in a concentration dependent fashion. The analysis of anaphase aberrations in embryos is useful because such an approach would aid in determining the source of micronuclei. Micronuclei may be a useful tool for detecting genotoxicants in the environment, however their etiology requires elaboration. Preliminary experiments with MIK indicate that cell death may also produce micronuclei in the apparent absence of lagging or broken chromosomes at anaphase, thought to be the route of micronuclei formation (see Heddle et al., 1983). Erythrocytic micronuclei incidence was correlated with anaphase aberration rates for EMS exposed embryos, and the assessment of erythrocytic micronuclei proved to be a more sensitive indicator of genotoxicity than AA analysis in similarly exposed organisms, possibly due to a higher mitotic rate in the erythropoietic tissue.

Observations of a large number of dead cells in 100 and 1000 mg/L EMS exposed embryos correlated with observations of teratogenesis, delayed mortality and multiple defects at anaphase. It is unclear whether the observed cell death was induced by a mutagenic or genotoxic event (EMS reacting principally with the DNA) or a toxicity induced event (EMS reacting with proteins, cellular macromolecules, including membranes). It was, however, apparent that cell death was present in concentrations exhibiting significantly higher levels of multiple damage, which weren't induced by MIK, indicating that EMS cytotoxicity may be through a genotoxic mechanism. The role of the observed cell death in delayed mortality and teratogenesis may be important, as non-genotoxic chemicals can induce cell death without inducing chromosome damage, and AA analysis may thus distinguish between genotoxicant induced teratogenesis and toxicant induced teratogenesis. The presence of induced cell death in concentrations inducing delayed mortality may indicate that normal maturation pathways of cells (for example erythrocytes) may have been disrupted, due to the reaction of EMS in dividing cell populations, or that certain enzymic development pathways were disrupted.

The sensitivity of embryo micronuclei and anaphase analysis was excellent, requiring only 24 hours exposure to demonstrate an effect at levels similar to those requiring weeks of exposure with adult fish. Some criticism of the AA technique, due to the high control levels of damage, is unwarranted, as levels of damage at anaphase were similar to those for corresponding metaphase lesions (Smith, 1984). The variety of detectable lesions at anaphase contrasts strongly with the limited observations possible at metaphase, and may enable investigators to detect mutagens other than those "breaking" chromosomes. These include spindle-fiber malfunctions and premetaphase alterations which can lead to aneuploidy (see Smith, 1984). The influence of cytochrome P-450s upon embryo sensitivity requires more study, however these enzymes are detectable and inducible prior to liver formation (Binder and Stegman, 1982) and analysis of erythrocytic micronuclei may combine the high enzyme activity of the liver with a high mitotic rate, possibly enhancing the sensitivity further. The implications of liver development upon the genotoxicity of direct-acting EMS require further study. The present study failed to expose embryos for the entire period up to liver formation, hence cannot fully answer this, however the considerably lower levels of AAs after 24 hours exposure, with analysis after liver formation as opposed to prior to formation, may indicate that detoxication of EMS is occurring. The embryo AA/micronuclei system may provide an excellent model for the study of hepatic versus extra-hepatic microsomal enzymes, and for determining the types of lesions resulting in micronuclei. The optimal assay for the detection of embryonic genotoxicants may include continuous exposure from fertilization, with sampling for analysis immediately prior to, and after, formation of the embryonic liver, and include both somatic AA analysis and erythrocytic MN analysis. Use of the embryo genotoxicity approach may provide valuable information on mutagenic or carcinogenic contaminants in wild fish populations, because many lipophilic carcinogens are expected to be passed to the offspring via the yolk material (Hose et al., 1981; 1983), and chemical uptake from the water can occur both during "water-hardening" and development. The subsequent observation of fish exposed as embryos to contaminants, or continually exposed beginning with embryos, may aid in determining the relative fate of lesions visible at anaphase, and interphase (micronuclei), and possibly provide altered (initiated) cells for the study of the promotion stage of carcinogenesis. The detection of genotoxicity at a concentration 1% of that estimated to be the safe level in an embryo-larval test points out the possible hazard in not considering the genotoxic effects of contaminants in discharges with low toxicity. The use of this approach for the screening of effluents and discharges for genotoxicity would provide a considerably larger safety margin for acceptable levels, if genotoxicants were present, than currently utilized approaches.

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IN VITRO FISH CELL DNA BREAKAGE AND REPAIR ASSAYS FOR DETECTING MUTAGENIC ACTIVITY

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In vitro short-term tests used with mammalian systems for detecting mutagenic activity have been refined for use with fish cells and activation enzymes. The results presented examine chromosomal damage and DNA repair in mammalian and fish cells following exposure to both model chemical mutagens and a series of polycyclic aromatic hydrocarbons.

ÉTUDES SUR LA RUPTURE ET LA RÉPARATION D'ADN DANS DES LES CELLULES DE POISSONS *IN VITRO* POUR DÉTECTER L'ACTIVITÉ MUTAGÈNE

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Des analyses *in vitro* à court terme, utilisées avec les systèmes mammifères pour déterminer l'activité mutagène, furent améliorées dans le but de les utiliser avec des cellules de poissons et des enzymes d'activation. Les résultats présentés examinent le dommage causé aux chromosomes et la réparation d'ADN dans des cellules de mammifères et de poissons faisant suite à l'exposition à des agents mutagènes chimiques modèles et à une série d'hydrocarbures aromatiques polycycliques.

INTRODUCTION

Surveys of fish populations from chemically contaminated areas have revealed an elevated frequency of tumours (Brown et al. 1973; Smith et al. 1979; Black et al. 1980, 1982). Chemical contaminants detected include polycyclic aromatic hydrocarbons such as benzo(a)pyrene, which has carcinogenic potential in fish (Brown et al. 1973; Black et al. 1980; Meyers and Hendricks 1982).

To further study problem areas, in vitro tests for mutagenic activity can be used. A plethora of such tests have been developed for mammalian systems usually employing rat activation enzymes and bacteria, yeast, rodent or human cells (Stich and San 1981). More recently, these cytogenetic techniques have been refined for use with fish cells and activation enzymes in order to compare mammalian assay results, determine fish cell sensitivity to mutagens, provide an assay with increased relevance to fish and the aquatic ecosystem and, ultimately, to develop a technique to directly assess the carcinogenic/mutagenic burden of a particular aquatic environment.

Since the genetic target of many carcinogens/mutagens is DNA, it is of both practical and theoretical significance to phyletically compare both DNA breakage and repair. The results of in vitro fish and mammalian cell DNA repair assays and chromosome breakage tests using both model mutagens and a series of polycyclic aromatic hydrocarbons are presented.

MATERIALS AND METHODS

Cell cultures

Stock cultures were grown in 75 cm^2 plastic flasks using MEM (Eagle's minimal essential medium) with 10% fetal calf serum, antibiotics and sodium bicarbonate (Walton et al. 1983). Mammalian lines were grown at 37°C in a water-saturated CO_2 incubator, while fish lines were kept in sealed flasks at 18°C. For experiments, cells were seeded onto coverslips and kept in growth medium until required.

DNA repair synthesis

Four to five days prior to chemical exposure, the coverslips were placed in ADM (arginine-deficient medium) with 2.5% serum in order to inhibit cell division (Walton et al. 1983). If not directly soluble in ADM with 2.5% serum, the test chemicals were initially dissolved in DMSO (dimethylsulfoxide) (maximum exposure concentration, 1-2%). A final ^{3}HTdR ([methyl-^{3}H]thymidine) concentration of 10 µCi/ml was obtained by dilution in ADM with 2.5% serum. One ml each of ^{3}HTdR and the test chemical were added for the treatment period. Where activation was required, 0.5 ml of an S9/cofactor mix was added to 0.5 ml of the test chemical and 1.0 ml ^{3}HTdR. Following the treatment period, the coverslips were washed with sodium citrate and then fixed in ethanol/acetic acid. Dried coverslips were mounted on slides with paraffin and processed autoradiographically using Kodak nuclear track emulsion (NTB-3). The amount of repair at a particular concentration of the test chemical was measured as the mean net grain count over at least 30 nuclei.

Chromosome aberration test

Following a 2-3 day growth period, coverslips of cells were exposed to 1.0 ml of the test chemical diluted in MEM with 2.5% serum (Walton et al. 1984). Where activation of the test chemical was required, 0.5 ml of an S9/cofactor mix was added to 0.5 ml of the test chemical. After a 3 h exposure, the coverslips were rinsed and the growth medium replaced to stimulate cell division. At 16 h post-exposure, cell division was inhibited using a 4 h colchicine treatment. The coverslips were then treated with KCl, fixed in ethanol/acetic acid, and stained with aceto-orcein. Chromosome aberrations (usually breaks and exchanges) were generally scored for 100 metaphases.

Micronucleus test

This tests follows the chromosome aberration test procedure, except that no colchicine was applied and series of coverslips were fixed and stained at intervals from 16 to 144 h following exposure to the test chemical. The broken DNA material forms micronuclei which were scored from examination of 500-1000 cells for each data point.

RESULTS AND DISCUSSION

Phyletic comparison of autoradiographically measured DNA repair (Table 1) indicates considerably more repair in mammalian versus fish cells. The repair measured peaked in all cell lines at approximately the same concentration of a particular chemical and, the magnitude of repair appears to be dependent on the chemical used. Between fish cell lines there was little variation in the repair magnitude despite cell lines originating from different species and tissues.

The fourfold difference in DNA repair between fish and mammalian cells cannot be accounted for on the basis of the quantity of DNA target since the fish cells have approximately only 10-15% less DNA per cell, as measured by microfluorometric detection of propidium iodide binding (D.G. Walton, unpublished data). A similar time course of repair has been found and,absorption of the test chemical appears not to be limiting since the same phyletic repair relationship is found following ultraviolet light exposure (Walton et al. 1983).

The DNA repair response of fish cells can be increased two to threefold by raising the assay temperature, lengthening the time for ^{3}HTdR incorporation, and increasing the ^{3}HTdR decay period (Walton et al. 1985). Using these enhanced assay conditions permitted the detection of repaired damage to fish cell DNA following exposure to polycyclic aromatic hydrocarbons (Table 2).

As the mammalian and fish cell lines examined had approximately the same DNA quality per cell, with the fish cells exhibiting markedly less repair, this suggests that considerable fish cell DNA damage may not be repaired; hence an assay that examines damage to the DNA, as opposed to repair, may be more sensitive.

Examination of chromosomal aberrations in fish cells (Table 3) showed a greater aberration frequency at lower chemical concentrations than in mammalian cells, but the peak frequency was found in Chinese hamster ovary cells. As with

Table 1. Autoradiographic DNA repair synthesis in mutagen-exposed human, rodent and fish cells[a,b]

	HF	CHO	RTG	RTO	CH	FHM
1. MNNG						
5×10^{-4}	75 ± 20	62 ± 10	27 ± 3.4	10 ± 2.3	22 ± 4.7	33 ± 4.6
1×10^{-4}	120 ± 29	64 ± 11	16 ± 2.1	9.1 ± 3.6	17 ± 4.1	25 ± 5.6
5×10^{-6}	17 ± 5.7	15 ± 2.8	2.4 ± 1.4	0.7 ± 0.9	1.2 ± 1.0	4.6 ± 2.7
2. 4NQO						
5×10^{-6}	231 ± 36	155 ± 10	23 ± 3.1	17 ± 2.7	17 ± 3.1	28 ± 2.9
1×10^{-6}	228 ± 36	140 ± 13	17 ± 1.4	11 ± 2.0	14 ± 1.8	14 ± 2.2
1×10^{-7}	91 ± 22	34 ± 11	7.6 ± 1.8	5.1 ± 1.9	9.3 ± 2.0	3.7 ± 2.1
3. NAAF						
5×10^{-5}	201 ± 9.1	88 ± 4.9	22 ± 4.0	12 ± 2.3	19 ± 2.5	16 ± 2.4
1×10^{-5}	125 ± 9.0	89 ± 15	9 ± 1.6	11 ± 2.1	13 ± 2.8	13 ± 2.8
1×10^{-6}	16 ± 2.1	20 ± 3.3	5 ± 1.0	2.5 ± 1.4	4.2 ± 1.6	2 ± 1.4
4. AFB$_1$						
a. With Rat S-9						
1×10^{-4}	115 ± 10	97 ± 6.1	18 ± 4.1	17 ± 2.4	5.7 ± 1.8	24 ± 4.2
5×10^{-5}	86 ± 11	92 ± 6.9	13 ± 2.9	12 ± 2.1	3.2 ± 1.6	22 ± 5.0
1×10^{-6}	3.5 ± 1.7	3.5 ± 1.3	2.2 ± 1.7	2.2 ± 1.9	0.9 ± 1.0	5.8 ± 1.5
b. Without Rat S-9						
1×10^{-4}	15 ± 3.1	11 ± 2.6	11 ± 1.8	1.1 ± 1.3	4.4 ± 1.4	5.8 ± 2.6
5×10^{-5}	9.8 ± 2.0	7.0 ± 2.1	10 ± 2.5	2.1 ± 2.0	3.7 ± 1.9	6.0 ± 2.4
1×10^{-6}	2.7 ± 1.7	1.1 ± 0.9	3.3 ± 1.7	1.7 ± 1.7	1.0 ± 1.1	1.0 ± 1.0
5. CONTROL						
	1.9 ± 1.5	1.9 ± 1.2	1.2 ± 1.0	1.5 ± 1.6	1.1 ± 1.3	1.1 ± 1.2

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[a] Mean nuclear grain counts ± standard deviation.

[b] **HF**, human fibroblast; **CHO**, chinese hamster ovary: **RTG**, rainbow trout gondad; **RTO**, rainbow trout ovary; **CH**, chum heart; **FHM**, fathead minnow; **MNNG**, N-methyl-N'-nitro-N-nitrosoguanidine; **4NQO**, 4-nitroquinoline 1-oxide; **NAAF**, N-acetoxy-2-acetylaminofluorene; **AFB$_1$**, Aflatoxin B$_1$.

Table 2. Autoradiographic DNA repair synthesis in enzyme activated polycyclic aromatic hydrocarbon exposed rainbow trout gonad cells using assay conditions which enhance the measured repair response[a,b]

Concentration (M)	Benzo(a)pyrene	1,2,5,6-Dibenzanthracene	1,2-Benzanthracene	Pyrene
2.5×10^{-4}	21(3)	5(2)	7(3)	2(1)
7.5×10^{-5}	19(3)	5(2)	1(2)	2(2)
1×10^{-6}	4(2)	2(2)	1(1)	3(2)
Control	4(2)	1(1)	2(2)	2(2)

[a] Mean net nuclear grain count ± standard deviation.

[b] Enzyme activation by arochlor 1254-exposed rainbow trout hepatic S9.

Table 3. Chromosome aberrations in human, rodent and fish cells exposed to chemical mutagens[a,b]

	HF	CHO	UI-H
1. MNNG			
5×10^{-4}	-	85	-
1×10^{-4}	29	17	-
7.5×10^{-5}	13	8	46
5×10^{-5}	5	5	33
5×10^{-6}	5	1	6
2. 4NQO			
7.5×10^{-6}	-	60	-
2.5×10^{-6}	25	8	-
1×10^{-6}	16	6	47
5×10^{-7}	8	1	36
1×10^{-7}	2	0	7
3. CONTROL			
	0	1	3

[a] Percent metaphases with chromosome aberrations.

[b] **HF**, human fibroblast; **CHO**, Chinese hamster ovary; **Ul-H**, Umbra limi heart; **MNNG**, N-methyl-N'-nitro-N-nitrosoguanidine, **4NQO**, 4-nitroquinoline 1-oxide.

Table 4. Chromosome aberrations in central mudminnow heart and Chinese hamster ovary cells exposed to enzyme activated polycyclic aromatic hydrocarbons[a,b]

Concentration (M)	Benzo(a)pyrene	1,2,5,6-Dibenzanthracene	1,2-Benzanthracene	Pyrene
Central mudminnow (*Umbra limi*) heart cells:				
7.5×10^{-5}	35	2	0	4
1×10^{-5}	10	6	1	0
1×10^{-6}	2	0	0	0
Control	2	1	2	0
Chinese hamster ovary cells:				
7.5×10^{-5}	41	0	0	1
1×10^{-5}	3	0	1	1
1×10^{-6}	1	1	2	1
Control	1	2	2	0

[a] Percent metaphases with chromosome aberrations.

[b] Enzyme activation by arochlor 1254-exposed rainbow trout hepatic S9.

Table 5. Micronuclei in human, rodent and fish cells exposed to chemical mutagens[a,b]

	HF	CHO	U1-H	U1-F
1. MNNG				
a. 16 h post-exposure				
5×10^{-5}	2.6	5.7	1.0	1.2
1×10^{-5}	2.7	1.3	1.6	1.3
5×10^{-6}	2.6	2.0	1.7	1.3
b. 80 h post-exposure				
5×10^{-5}	2.7	18.8	7.9	3.7
1×10^{-5}	2.6	13.4	3.0	2.6
5×10^{-6}	2.1	6.6	2.2	0.8
c. 144 h post-exposure				
5×10^{-5}	3.2	18.2	9.6	4.6
1×10^{-5}	2.6	7.0	4.0	3.5
5×10^{-6}	2.4	3.0	2.8	3.0
CONTROL				
	1.1	0.9	1.4	1.1
2. 4NQO				
a. 16 h post-exposure				
1×10^{-6}	2.2	2.3	2.3	0.7
5×10^{-7}	2.8	7.5	1.1	1.1
5×10^{-8}	2.2	1.8	1.0	0.9
b. 80 h post-exposure				
1×10^{-6}	3.0	9.4	5.4	1.5
5×10^{-7}	3.3	6.9	5.4	1.5
5×10^{-8}	1.6	1.2	2.3	1.2
c. 144 h post-exposure				
1×10^{-6}	3.0	3.2	7.8	1.8
5×10^{-7}	2.6	2.0	7.0	1.6
5×10^{-8}	2.3	1.9	3.2	1.4

Table 5 cont...

	HF	CHO	Ul-H	Ul-F
2. 4NQO 144 h post-exposure cont...				
CONTROL				
	1.7	1.1	1.8	1.1

[a] Percent micronuclei.

[b] **HF**, human fibroblast; **CHO**, Chinese hamster ovary; **Ul-H**, *Umbra limi* heart; **Ul-F**, *Umbra limi* fin; **MNNG**, N-methyl-N'-nitro-N-nitrosoguanidine; **4NQO**, 4-nitroquinoline 1-oxide.

the DNA repair response following polycyclic aromatic hydrocarbon exposures, positive chromosome aberration results were only detected following exposure to benzo(a)pyrene (Table 4).

Allowing the cells with broken chromosomal material to divide results in the formation of small micronuclei. The micronuclei test results (Table 5) parallel the chromosomal data, but the frequency of micronuclei appears to be dependent on the rate of cell division. The slower dividing human fibroblast and *Umbra limi* fin cells have correspondingly lower micronucleus frequencies, perhaps due, in part, to a longer time to repair DNA damage prior to cell division.

SUMMARY

The low amount of fish cell DNA repair will likely preclude the assay as a viable aquatic mutagenicity test, but it suggests that assays which examine damage to DNA directly may be more sensitive. Examination of the frequency of fish cell chromosomal aberrations appears to be a relatively sensitive technique, but few fish species have low chromosomal complements which facilitates easy analyses. Although dependent on the rate of cell division, the micronucleus test also appears to be fairly sensitive and is not dependent on chromosome numbers. This assay with fish cells, both *in vitro* and *in vivo*, may therefore be the most useful and widely applicable.

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THE FISH - THE ULTIMATE CARCINOGEN ASSAY

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The potential of fish as useful test organisms for detecting carcinogens in the environment will be discussed.

In the environment, cancer-causing chemicals can be identified by correlating data from four bases. These four data bases, i.e. human cancer epidemiology, bioassays using either short-term in vitro tests or long-term whole animal studies, environmental analytical studies to assess population exposures and the documentation and study of epizootics of neoplasia in fish and/or wildlife populations, all have potential to aid in the identification of environmental carcinogens. In this systems approach, fish may occupy a unique position in that they can serve as both laboratory animals for carcinogen bioassays and/or experimental models of chemically induced neoplasia. Many fish species are "real world" organisms and some species appear to have the potential to serve as effective in place monitors or sentinel organisms for detecting water-borne carcinogens.

Although many wild fish species may have this capacity, the brown bullhead (Ictalurus nebulosus) has been shown to be a sensitive and useful species. Recent experiments conducted in our laboratory have shown the induction of skin and liver neoplasms in brown bullheads in response to a complex mixture of organic chemicals isolated from polluted river sediments (Buffalo River, Erie County, New York). Brown bullheads collected from the river exhibit histologically similar types of neoplasms.

In addition, a number of domesticated fish species have shown considerable promise as cost effective experimental models/bioassays of chemically induced carcinogenesis. Promising species include several small aquarium species such as the medaka (Oryzios latipes), zebra fish (Brachydanio rerio), guppy (Lebistes reticulatis) and the larger rainbow trout (Salmo gairdneri). Two novel embryo exposure methods (to be described) include the direct injection of chemical agents into trout embryos as eyed-stage eggs, as well as a novel passive chemical absorption technique. The advantage of these two exposure methods will be compared. Their utility offers the chemical toxicology researcher interested in chronic health effects a new fish assay(s) with considerable potential for multi-stage, multi-endpoint chemical testing.

LE POISSON - L'ESSAI CANCÉRIGÈNE ULTIME

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L'utilisation possible des poissons en tant qu'organismes détecteurs et indicateurs de substances cancérigènes dans l'environnement est discutée.

Dans l'environnement, les agents chimiques causant le cancer peuvent être identifiés à l'aide de corrélations entre données provenant de quatre sources. Ces quatre sources sont: l'épidémiologie du cancer humain; des bioassais utilisant soit des expériences à court terme *in vitro* ou des études à long terme sur des animaux; des études analytiques environnementales estimant l'exposition de populations; et les études épizootiques de néoplasies dans les poissons et/ou dans la faune. Toutes ces données offrent une aide potentielle pour l'identification des substances cancérigènes dans l'environnement. Les poissons peuvent occuper une position unique dans cette approche de systèmes car ils peuvent servir comme animaux de laboratoire pour les bioassais d'agents cancérigènes et/ou comme modèles expérimentaux d'induction chimique de néoplasie. Plusieurs espèces de poissons sont des organismes "réels" et plusieurs espèces paraissent avoir le potentiel pour servir efficacement comme moniteurs ou organismes sentinelles pour détecter les agents cancérigènes transportés par voie d'eau.

Bien que plusieurs espèces de poissons aient cette capacité, la barbotte brune (*Ictalurus nebulosus*) s'est révélée une espèce particulièrement utile. Des expériences conduites récemment dans notre laboratoire ont abouti à l'induction de néoplasmes dans la peau et dans le foie de barbottes brunes en réponse à un mélange complexe de produits chimiques organiques isolés à partir de sédiments d'une rivière polluée (Buffalo River, Erie County, New York). Des barbottes brunes provenant de la même rivière ont des types de néoplasmes similaires.

De plus, plusieurs espèces de poissons domestiques ont un potentiel prometteur pour garder bas le coût de modèles expérimentaux/bioassais d'induction chimique de carcinogénèse. Ces espèces comprennent plusieurs petits poissons d'aquarium tels le néant (*Oryzios latipes*), le poisson zèbre (*Brachydanio rerio*), le guppy (*Lebistes reticulata*) et des plus grosses espèces, telle la truite arc-en-ciel (*Salmo gairdneri*). Deux nouvelles méthodes d'exposition d'embryons (qui seront décrites plus tard) sont l'injection directe d'agents chimiques dans l'embryon de la truite au stage où l'on peut discerner les yeux sur les oeufs et aussi une nouvelle technique d'absorption chimique passive. Les avantages de ces deux méthodes d'exposition seront comparés. Leur utilité offre aux chercheurs en toxicologie chimique intéressés aux effets de santé chroniques de nouveau(x) bioassai(s) avec poissons ayant un potentiel considérable pour des essais à plusieurs étapes et à aboutissements multiples.

PATHOLOGICAL ANOMALIES IN CONTROL FISH: SEVERAL CASE STUDIES

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A review of several cases from toxicological or nutritional trials revealed several instances of major pathological changes in control (and often all) fish. Alterations in gill morphology especially are of major importance for aquatic toxicology. Several instances of parasitism and infection raised doubts about the "normal" status of fish in behavior and toxicological trials, including contaminant uptake and excretion in body tissues. A review of these instances highlights the importance of ascertaining that the test population is indeed "normal" prior to the initiation of experiments.

ANOMALIES PATHOLOGIQUES DANS DES POISSONS TEMOINS: PLUSIEURS ETUDES DE CAS

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Une revue de plusieurs cas pris à partir d'études en toxicologie ou en nutrition révéla plusieurs occasions où des changements pathologiques majeurs apparurent dans les poissons témoins (et souvent dans tous les poissons). Les altérations dans la morphologie des branchies sont surtout d'importance majeure pour la toxicologie aquatique. Plusieurs occasions de parasitisme et d'infection ont soulevé des doutes à propos du statut 'normal' des poissons dans les essais en comportement et en toxicologie, ceci incluant l'absorption et l'excrétion d'agents contaminants dans les tissus corporels. Une revue de ces circonstances souligne l'importance de s'assurer que la population testée est en effet 'normale' avant l'initiation des expériences.

A COMPUTERIZED AQUATIC RESPIROMETER FOR SUBLETHAL STUDIES

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A computer-controlled respirometer has been designed to measure the effects on fish respiration rates of exposure to sublethal concentrations of potential pollutants. The system is a semi flow-through type with 8 respirometer chambers. Miniature laser transmitters and receivers mounted on the side of each chamber provide the capability of monitoring organism activity. At 16 bit, 64K minicomputer, with a real time clock, uses custom electronics to interface with a series of solenoid valves. Software allows user control of water flow and toxicant concentration and delivery via the valves. Control of length of pre-exposure, exposure and recovery periods is also provided. The computer receives data from 4 polarographic oxygen probes, 4 temperature probes and the activity monitoring system. These data are sorted and stored on floppy disk and printed at regular intervals. The system can accommodate both marine and freshwater organisms.

Rainbow trout (Salmo gairdneri) with a mean weight of 230 g were exposed to acephate (0,S-dimethylacetylphosphoroamidothiate), a water soluble organophosphorus insecticide used in the control of forest pest insects. After a 2-day acclimation, fish were exposed to acephate for 3 days followed by a 3-day post-exposure period. After 2 days exposure to 37 or 60 ppm, fish exhibited a significant increase in respiration compared to controls ($p<0.001$). Respiration rates of exposed fish approached those of control fish 2-3 days into recovery. No significant differences were observed at 6 or 21 ppm. Activity did not change as a function of acephate concentration and was used to correct respiration rates for differences in spontaneous activity.

The increased respiration rates at higher acephate concentrations and subsequent recovery corresponds well with earlier uptake and elimination experiments which showed that uptake was proportional to acephate concentration and elimination was rapid (50% elimination in ≈ 2 days).

UN RESPIROMETRE AQUATIQUE INFORMATISE POUR DES ETUDES SUB-LETALES

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Un respiromètre contrôlé par ordinateur a été conçu pour mesurer les effets de l'exposition à des concentrations sub-létales de substances pontiellement polluantes sur la respiration des poissons. La système est de type à écoulement partiel avec 8 chambres à respiromètre. Des transmitteurs et receveurs à laser miniaturisés et montés sur le côté de chaque chambre permettent de suivre l'activité des organismes. Un miniordinateur à 16 bit et 64K, avec une montre, utilise des commandes électroniques pour faire interface avec une série de valves solénoides. Le logiciel permet à l'utilisateur de contrôler l'écoulement d'eau et la concentration et l'arrivée de substances toxiques par les valves. Le contrôle de la durée de préexposition, d'exposition et des périodes de récupération est aussi possible. L'ordinateur reçoit les données de 4 sondes polarographiques d'oxygène, de 4 sondes de température et du système de contrôle d'activité. Ces données sont classées et entreposées sur des disques flexibles et imprimées à des intervalles réguliers. Le système peut accommoder les organismes d'eau douce et marins.

Des truites arc-en-ciel (Salmo gairdneri) ayant un poids moyen de 230 g furent exposées à de l'acéphate (0-S-diméthylacétyl phosphoroamidothiate), un insecticide organophosphoré soluble dans l'eau et utilisé dans le contrôle des insectes nuisibles en forêt. Après une période d'acclimatation de 2 jours, les poissons furent exposés à l'acéphate pour 3 jours, après quoi il y eut une période de post-exposition de 3 jours. Après deux jours d'expositiion à 37 ou 66 ppm, les poissons montrèrent une augmentation significative dans leur respiration, comparée à celle des témoins ($p<0.001$). Le taux de respiration des poissons exposés approchait celui des poissons témoins après 2-3 jours de récupération. On n'observa aucune différence significative à 6 ou à 21 ppm. L'activité ne changea pas en fonction des concentrations d'acéphate et fut utilisée pour corriger le taux de respiration pour les différences dans l'activité spontanée.

L'augmentation du taux de respiration à de hautes concentrations d'acéphate et la récupération subséquente sont en accord avec les expériences antérieures d'absorption et d'elimination qui montraient que l'absorption était proportionnelle à la concentration d'acéphate et que l'élimination était rapide (élimination de 50% en ≈ 2 jours).

TOXICOKINETICS OF CHLORINATED PHENOLS IN RAINBOW TROUT FOLLOWING DIFFERENT ROUTES OF CHEMICAL ADMINISTRATION

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Chlorinated phenols (CPs) are chemicals which are widely used as wood preservatives in industry. Previous studies have shown that CPs are frequently found in water samples and are very toxic to aquatic organisms.

In the present study, we examined the toxicokinetics of 2,4-dichlorophenol (DCP), 2,4,6-trichlorophenol (TCP) and 2,3,4,6-tetrachlorophenol (TTCP) in rainbow trout implanted surgically with a cannula in the dorsal aorta. Blood samples were withdrawn from the cannula at various time points after a fish was exposed to individual CPs in water (0.1 - 0.5 ppm) or was given CPs by the oral (50 mg/kg) or intravenous route (10 mg/kg).

Following intravenous administration of CPs to trout, unchanged CP concentration in the blood was found to decline biphasically with time. The biological half-lives for DCP, TCP and TTCP were, respectively, 78 min, 74 min and 87 min. When trout were exposed to water containing individual CPs, the blood concentration vs time curves could be described by a biexponential equation. The data was fitted to a one-compartment model. The absorption rate constants estimated for DCP, TCP and TTCP were, respectively, 0.04 min^{-1}, 0.03 min^{-1} and 0.01 min^{-1}. In contrast, following oral administration of CPs to trout, very low levels of unchanged CPs were detected in the blood, indicating a slow rate of absorption by this route of exposure.

This study indicates that CPs are absorbed rapidly via the gill of the trout and that the chemicals were, in order of increasing rates of elimination, TTCP, DCP and TCP.

LA CINÉTIQUE TOXICOLOGIQUE DE PHÉNOLS CHLORÉS CHEZ DES TRUITES ARC-EN-CIEL SELON DIFFÉRENTES VOIES D'ADMINISTRATION CHIMIQUE

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Les phénols chlorés (PCs) sont des produits chimiques fréquemment utilisés comme agents de conservation du bois dans l'industrie. Des études antérieures ont démontré que les PCs sont fréquemment rencontrés dans les échantillons d'eau et qu'ils sont très toxiques pour les organismes aquatiques.

Dans cette étude, nous avons examiné les mouvements du 2,4-dichlorophénol (DCP), du 2,4,6-trichlorophénol (TCP) et du 2,3,4,6-tétrachlorophénol (TTCP) à l'intérieur de truites arc-en-ciel ayant été chirurgicalement munies de canules dans l'aorte dorsale. Des échantillons de sang furent pris à partir de canules à certains intervalles après qu'un poisson ait été exposé à des PCs individuels dans l'eau (0.1-0.5 ppm) ou ait été administré des PCs par voie orale (50 mg/kg) ou par voie intraveineuse (10 mg/kg).

Après l'administration intraveineuse des PCs aux truites, on nota que la concentration de PC non-transformé dans le sang varie bimodalement dans le temps. Les demi-vies biologiques du DCP, TCP et TTCP étaient respectivement de 78, 74 et 87 min. Quand les truites furent exposées à de l'eau contenant des PCs individuels, les courbes de la concentration dans le sang en fonction du temps pouvaient être décrites par une équation biexponentielle. Les données furent ajustées à un modèle à un compartiment. Les constantes de taux d'absorption estimées pour le DCP, TCP et TTCP étaient de 0.04, 0.03 et de 0.01 min.^{-1} respectivement. Par comparaison, après que les PCs aient été administrés oralement aux truites, on détecta de très bas niveaux de PCs non-transformés dans le sang, ce qui indique un taux d'absorption très bas à travers cette forme d'exposition.

Cette étude indique que les PCs sont rapidement absorbés à travers les branchies des truites et que les produits chimiques éliminés le plus rapidement sont, du plus lent au plus rapide, le TTCP, DCP et TCP (subventionné par le CRSNGC).

DISEASES OF MARINE FISHES IN PUGET SOUND AND RELATIONSHIPS TO POLLUTION

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Over the past decade, we have conducted a series of field studies concerned with the presence of diseases in bottom-dwelling fishes in Puget Sound and possible relationships to sediment-associated chemicals. A variety of pathological conditions have been found in English sole (Parophrys vetulus), starry flounder (Platichthys stellatus), rock sole (Lepidopsetta bilineata), and Pacific staghorn sculpin (Leptocottus armatus). Some conditions were associated with infectious agents (e.g., parasites and microorganisms), and some were idiopathic (i.e., of unknown etiology). Fish with infectious conditions and with certain types of idiopathic abnormalities were widely distributed in both urban and nonurban embayments. However, other types of idiopathic lesions, especially of the liver, were found primarily in the four fish species from polluted urban embayments. These lesions were classified into four major categories: neoplasms, hyperplasia/foci of cellular alteration, megalocytic hepatosis, and steatosis/hemosiderosis. The prevalences of these lesions varied considerably among species within the same embayment; for example, the highest prevalences of hepatic neoplasms in English sole (16.2%) and starry flounder (1.1%) were both found in the same waterway. Concentrations of selected metals and aromatic hydrocarbons in sediments were positively correlated with the prevalences of some of the liver lesions, including neoplasms. Although these correlations do not provide definitive evidence of cause and effect, they do provide clues of presumptive relationships between chemicals and liver lesions which are presently being evaluated in laboratory studies.

MALADIES DE CERTAINS POISSONS MARINS DANS PUGET SOUND EN RELATION À LA POLLUTION

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Durant la dernière décennie, nous avons conduit une série d'études sur la présence de maladies chez les poissons de fond de Puget Sound et sur leurs relations possibles avec les produits chimiques associés aux sédiments. Nous avons trouvé plusieurs conditions pathologiques dans la sole anglaise (Parophrys vetulus), la plie du Pacifique (Platichthys stellatus), la sole du Pacifique (Lepidopsetta bilineata) et dans le chabot armé (Leptocottus armatus). Quelques unes de ces conditions étaient liées aux agents infectieux (e.g. parasites et microorganismes), et d'autres étaient idiopathiques (i.e. d'étiologie inconnue). Les poissons avec des conditions infectieuses et avec certains types d'anormalités idiopathiques étaient largement distribués dans les anses urbaines et non urbaines. Cependant, d'autres types de lésions idiopathiques, surtout dans le foie, furent trouvées en grande partie dans les quatre espèces de poissons provenant d'anses urbaines polluées. Ces lésions furent classifiées en quatre catégories principales: néoplasmes, hyperplasie/foyers d'altérations cellulaires, hépatoses mégalocytiques, et stéatoses/hémosidéroses. La fréquence de ces lésions variait considérablement parmi les espèces provenant de la même anse; par exemple, les plus hautes fréquences de néoplasmes dans la sole anglaise (16.2%) et dans la plie du Pacifique (1.1%) furent toutes deux trouvées dans le même cours d'eau. Les concentrations de certains métaux et d'hydrocarbures aromatiques dans les sédiments furent corrélées positivement avec la fréquence de certaines lésions hépatiques, incluant les néoplasmes. Bien que ces corrélations ne fournissent pas d'évidence définitive de cause et d'effet, elles fournissent des indices quant aux relations entre les produits chimiques et les lésions hépatiques qui sont présentement étudiées en laboratoire.

CETIS: COMPLEX EFFLUENTS TOXICITY INFORMATION SYSTEM

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A computerized Complex Effluent Toxicity Information System (CETIS) data base has been designed to assemble the results of effluent toxicity tests so that toxicity characteristics of effluents on an industry-by-industry basis can be determined. The information is available to state and regional environmental offices to assist them in determining where to use toxicity testing, in interpreting the results, and in setting discharge limits.

Data for CETIS are obtained both from published papers and from unpublished results of tests conducted by region or state agencies. The data base currently includes information from 16 states in the U.S. and from Canada. These data are then evaluated by trained reviewers, selected information is encoded onto data record forms, and encoded data are entered into the computer data base stored at the National Computer Center. A template retrieval system is available that includes selection criteria and sort options to provide the following categories of information: [1] industry report, [2] receiving water or area report, [3] test report, [4] test species report, [5] effluent treatment report, and [6] total data listing.

SITEC: SYSTÈME D'INFORMATION SUR LA TOXICITÉ D'EFFLUENTS COMPLEXES

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Une base de données sur un Système d'Information sur la Toxicité d'Effluents Complexes (SITEC) fut assemblée sur ordinateur pour grouper les résultats de tests de toxicité d'effluents pour que les caractéristiques toxiques d'effluents puissent être déterminées pour chaque type d'industrie. L'information est accessible aux bureaux régionaux et d'état en vue de les aider à déterminer où tester pour la toxicité, à interpréter les résultats et à fixer les limites de décharge.

Les données pour SITEC sont obtenues à partir d'articles publiés et à partir de résultats non publiés de tests ayant été conduits par des agences régionales ou d'état. La base des données inclut présentement de l'information provenant de 16 états des É-U. et du Canada. Ces données sont ensuite évaluées par des réviseurs spécialement entraînés. L'information choisie est encodée sur des formulaires d'inscription de données, et les données encodées sont entrées dans la base de données sur un ordinateur situé dans le Centre National d'Informatique. On peut utiliser un système d'accès incluant les critères de sélection et triant les options qui peut fournir les types d'information suivants: [1] rapports sur les industries, [2] rapports sur les eaux contaminées et leur région, [3] rapports sur les tests performés, [4] rapports sur les espèces testées, [5] rapports sur le traitement des effluents, et [6] liste de toutes les données.

CARCINOGENESIS IN WILD FISH POPULATIONS

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Neoplastic disease in some populations of wild fish may be examples of environmental carcinogenesis. Support for this conclusion relies heavily on the inference that either a high prevalence or increasing incidence of neoplasms, in a particular species or local population, means that chemical carcinogens are responsible. Since many factors contribute to neoplasia in animals of all types, including fish, this concept that a direct link exists between environmental contamination and neoplasms in fish must be examined carefully. Consideration must be given to factors such as virus, genetic immune dysfunction, habitat and chance. If these complex interactions are ignored, the conclusion that the high prevalence of neoplasms in some fish populations results solely from chemical contamination of the fishes environment may be misleading. Examples of each of these interactions will be discussed.

The use of the term "cancer" for the description of tumorous conditions in fish is also misleading. Cancer by definition refers to "a cellular tumor the natural course of which is fatal", and "unlike benign tumor cells, exhibit the properties of invasion and metastasis and are highly anaplastic" (Dorlands). Rarely are such conditions seen in either wild or cultured fish. The neoplastic growths commonly seen in fish are benign. The misuse of the terminology used to describe neoplastic growths in fish will also be discussed.

CARCINOGÉNÈSE DANS DES POPULATIONS SAUVAGES DE POISSONS

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Les maladies néoplasiques de certaines populations de poissons sauvages peuvent être des exemples de carcinogénèse environnementale. Le support pour cette conclusion dépend fortement de l'inférence qu'une forte prédominance ou qu'une augmentation dans la fréquence des néoplasmes dans une espèce particulière ou dans une population locale, signifie que des produits chimiques cancérigènes sont à l'oeuvre. Etant donné que plusieurs facteurs contribuent à la néoplasie dans les animaux de tous types, ce concept, qui veut qu'un lien direct existe entre la contamination environnementale et les néoplasmes dans les poissons, doit être examiné attentivement. On doit considérer les facteurs tels les virus, une malfonction génétique immunitaire, l'habitat et la chance. Si ces interactions complexes sont ignorées, la conclusion que la haute fréquence des néoplasmes dans une population de poissons résulte seulement de la contamination chimique du milieu aquatique peut être trompeuse. Des exemples de chacune de ces interactions seront discutés.

L'utilisation du terme 'cancer' pour décrire des conditions de tumeurs chez les poissons est aussi trompeuse. Par définition, cancer réfère à "une tumeur cellulaire dont l'aboutissement naturel est fatal" et qui "contrairement aux cellules de tumeur bénigne, montre des propriétés d'invasion et de métastase et est hautement anaplasique" (Dorlands). De telles conditions sont rarement rencontrées chez les poissons en culture ou sauvages. Les croissances néoplasiques communément rencontrées chez les poissons sont bénignes. La mauvaise utilisation de la terminologie employée pour décrire l'accroissement néoplasique chez les poissons sera aussi discutée.

PETROLEUM AND POLLUTION

PAPERS AND ABSTRACTS

USE OF BIOASSAY TECHNIQUES TO EVALUATE THE EFFECTIVENESS OF NATURAL AND CHEMICAL DETOXIFICATION OF TAR SANDS TAILINGS WATERS

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During the production of synthetic crude oil from the Athabasca Oil Sands, Syncrude Canada Ltd. produces 20-30x10^6 m^3 of waste water each year. The water, stored in a 13 km^2 tailings pond, is acutely toxic, with the 96 h LC_{50} values based on static bioassays with trout and Daphnia being less than 10%. With the Beckman Microtox bioassay, the EC_{50} (concentration of test solution which causes a 50% reduction in the light output of the bioluminescent bacteria used in the assay) is 20-30%. The Microtox technique, which gives results in 15 minutes and requires only 5 ml of sample, has been useful in screening the effectiveness of various chemical treatments of tailings water, as well as following its rate of detoxification during storage. The most effective treatment of tailings water found so far involves flocculation of the suspended solids under acidic conditions. When the clear supernatant is decanted and neutralized, trout and Daphnia can survive in the water for 96 hours and the Microtox test shows no reduction in bacterial bioluminescence. Studies with 300 m^3 experimental pits have shown that naturally-occurring physical, chemical and biological processes during storage can result in tailings water which is not acutely toxic within as short as 14 months. A four-stage sequential testing procedure involving the Beckman Microtox Test, the 96-hour static trout test and biomonitoring has been found to be effective in the development of wastewater treatment programs.

L'UTILISATION DE TECHNIQUES DE BIOASSAI POUR ÉVALUER L'EFFICACITÉ DE DÉSINTOXIFICATION NATURELLE ET CHIMIQUE DES EAUX DE DÉCHÊTS DES SABLES BITUMINEUX

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Durant la production de pêtrole synthétique brut provenant des sables bitumineux d'Athabaska, Syncrude Canada Ltée produit de 20 à 30 x 10^6 m^3 d'eaux résiduaires chaque année. Cette eau, gardée dans un bassin de 13 km^2, est intensément toxique, ayant une valeur de CL_{50} pour 96 heures de moins de 10%, basée sur des bioassais statiques faits avec des truites et des daphnies. Avec le bioassai Microtox de Beckman, le CE_{50} (concentration d'une solution testée qui cause une réduction de 50% de la lumière produite par la bactérie bioluminescente utilisée dans l'essai) est de 20 à 30%. La technique Microtox donne des résultats en dedans de 15 minutes et requiert seulement un échantillon de 5 ml. Cette technique est utile pour identifier l'efficacité de divers traitements chimiques des eaux de rebut, ainsi que pour en suivre le taux de désintoxification durant l'entreposage. Le traitement d'eaux usées le plus efficace jusqu'à date implique la floculation des solides suspendus sous des conditions acidiques. Quand le supernatant clair est décanté et neutralisé, les truites et daphnies peuvent survivre dans l'eau pour 96 heures et le test Microtox ne montre aucune réduction dans la bioluminescence bactérienne. Des études menées avec des puits expérimentaux de 300 m^3 ont montré que l'apparition naturelle de processus physiques, chimiques et biologiques durant l'entreposage peut résulter en des eaux de rebut qui ne sont plus intensément toxiques en dedans de 14 mois. Un procédé d'analyse séquentielle en quatre étapes utilisant le test Microtox de Beckman, l'analyse statique de 96 heures avec truites et la supervision biologique s'est révélé efficace dans le développement de programmes de traitements des eaux résiduaires.

INTRODUCTION

Syncrude Canada Ltd. is designed to produce over 40 x 10^6 barrels of synthetic crude oil annually from the Athabasca Oil Sands near Fort McMurray in northeastern Alberta. A caustic hot water process is used to extract the bitumen from the oil sand, followed by the upgrading of the bitumen through coking and hydrotreating to produce synthetic crude oil. Over 100 x 10^6 m^3 of waste water and sludge are produced annually and are stored in a tailings pond. However, most of the water is recycled, resulting in a net annual accumulation of 20 - 30 x 10^6 m^3 of waste water and sludge.

By mid-1984, the tailings pond contained 180 x 10^6 m^3 of waste water and sludge, covered an area of 13 km^2 and had a maximum depth of 35 m (Figure 1). The pond is stratified into a surface water zone (0 - 10 m) with a low concentration of solids (less than 1%) and a deeper sludge zone containing 5 - 45% solids. The tailings pond water is acutely toxic, with 96 h LC_{50} values for trout and Daphnia being less than 10%. Bacteria (mainly Alcaligenes spp. and Acinetobacter spp.) appear to be the only organisms present in the pond.

Under the present zero-discharge policy, Syncrude's tailings pond is projected eventually to cover 17 km^2, have a maximum depth of 60 m and contain over 400 x 10^6 m^3 of wastewater and sludge. Current reclamation options provide for either reclaiming the pond in situ as a viable, non-toxic water body, or of treating and discharging the wastewater in an environmentally acceptable manner and revegetating the disturbed land area.

MacKinnon and Retallack (1982) examined the effectiveness of various chemical and physical treatments in detoxifying tailings pond water. The only method found which resulted in a high degree of detoxification involved coagulation of the suspended solids under acidic conditions, with subsequent separation and neutralization of the clarified water. The objective of this paper is to provide further data showing that rapid detoxification of tailings water can be accomplished by acidification and that the effectiveness of this treatment can be improved by incorporation of a suitable flocculant. We also describe the naturally-occurring detoxification of tailings pond water stored in experimental pits free from fresh tailings input since June 30, 1983. We have evaluated both the short-term chemical treatment and the long-term natural detoxifiction using various acute bioassays. Experience with these bioassays led to the development of a sequential testing procedure which we feel has application in development of treatment plans for other toxic wastewaters.

METHODS

Chemical treatments

In May, 1984, three different chemical treatments of tailings were evaluated: coagulation of the suspended solids with acid (Treatment 1), coagulation with acid followed by addition of a flocculant (Treatment 2), and coagulation with alum followed by addition of a flocculant (Treatment 3). These treatments were chosen in order to assess the effectiveness of the flocculant (Treatment 1 vs 2) and the importance of acid as the coagulant (Treatment 2 vs 3).

Tests were carried out in polyethylene-lined barrels containing 200 litres of water collected from 25 cm below the surface of the tailings pond and allowed to stand for one week at 20°C to remove volatiles. Treatment 1 consisted of slowly adding 1 M H_2SO_4 under constant agitation until the pH was reduced from 8.1 to 4.5 (approximately 12 meq/L of acid). Treatment 2 consisted of acidification to pH 4.5 followed by the addition of a 5 ppm high molecular weight, medium charge density, anionic polyelectrolyte (CFA30, manufactured by Crossfield Polyelectrolytes, U.K.) under mild agitation. In Treatment 3, alum ($Al_2(SO_4)_3$ as a 10 mg/L stock solution) was added to give a final concentration of 100 ppm. A fourth barrel of tailings water used as a control was agitated to a similar degree as the water in the three treatment barrels.

After 24 hours, samples were taken from all four barrels and pH adjusted to 8.0 with 1 M NaOH. In order to compare the rate of clarification, samples for suspended solids were also taken 0.25, 1, 2, 24 and 48 h after treatment. Physical and chemical analyses and toxicity tests were carried out as described below. For comparison, a natural water sample taken from the Athabasca River just upstream from Syncrude's pumping station was included in the analysis. This sample was intended as a reference against which to compare the untreated tailings pond water and the waters produced by the three treatments.

Ageing of tailings pond water

Two experimental pits were excavated adjacent to the tailings pond. Each pit measured 13 m by 13 m at the surface, had walls with a 1:1 slope, a maximum depth of 3 m in the central area and a volume of 300 m^3. Each pit was lined with a 10 mil sheet of nylon-reinforced polyethylene. On June 30, 1983, the pits were filled with water from the surface zone of the tailings pond. The chemistry and toxicity of the water in the pits was monitored at 1 - 3 month intervals.

Physical and chemical analysis of water samples

Dissolved oxygen, conductivity and pH were measured with a Hydrolab meter calibrated prior to use. Total solids were determined gravimetrically after drying 12 - 20 ml of whole sample at 100°C. Dissolved solids were determined gravimetrically after removal of suspended solids by filtration through a 0.8 μm Millipore filter. Suspended solids were determined by difference. Turbidity was measured with a Hach Model 2100 Turbidity Meter. Analysis of other chemical parameters followed standard procedures as described by Alberta Environment (1978). Minor elements were analyzed by atomic absorption.

Toxicity tests

The Beckman Microtox method was used for routine screening of water samples for toxicity. This is essentially a 15-minute static bioassay using a bioluminescent bacterium as the assay species. The amount of light produced by bacteria, as measured with a luminometer, is inversely proportional to the toxicity of the solution being tested (Bulich et al. 1981). Tests were carried out as described in Beckman Instruments Interim Manual No. 110679B-9-80.

Ninety-six hour static toxicity tests were carried out with rainbow trout as described by Alberta Environment (1978). Each test consisted of five effluent concentrations plus a control. Five or ten fish (0.5 - 1.0 g) were tested in 20 - 25 litres of each concentration.

Static acute (96 h) tests were also conducted with Daphnia magna. The acute test involved in five concentrations (0, 25, 50, 75 and 100%) with five animals being exposed to 100 ml of each concentration. Each concentration was replicated three times (total of 75 animals).

RESULTS AND DISCUSSION

a) Evaluation of chemical treatments

In Table 1 the physical, chemical and toxicological properties of the waters resulting from the three chemical treatments (T1, T2, T3) are compared with untreated tailings pond water (TP) and Athabasca River water (AR). Both the acid and the alum in combination with flocculant (T2 and T3) resulted in greater than 95% removal of suspended solids from the tailings pond water, with a decrease from over 1000 mg/L to 20 mg/L in 24 hours. Turbidity also decreased from 680 NTU to about 30 NTU. The major effect of the flocculant was to increase the rate of clarification (Figure 2).

With all three treatments there was about a 75% reduction in color (from 1,000 TCU to 200 - 300 TCU), most likely due to the reduction in tannin and lignin concentrations from 9 mg/L to about 4 mg/L. All treatments also resulted in signficant reductions in COD (30 - 55%) and BOD (40 - 60%). As expected, the addition of H_2SO_4 and NaOH during Treatments 1 and 2 resulted in increases in conductivity, sulphate and sodium in these samples.

Although removal of suspended solids can be achieved with either alum or acid as coagulant, only acidification results in detoxification to a level where values of EC_{50} (for Microtox) and LC_{50} (for fish and Daphnia) are over 100%. The reduction in toxicity with alum as determined with Daphnia (increase in LC_{50} from 2% to 38%) is quite likely related to the removal of suspended solids, high concentrations of which clog the filtering mechanism of the animals (MacKinnon and Retallack 1982). Alum treatment did not result in any detoxification when tested with Microtox or fish.

Potentially toxic substances present in the tailings pond water showed various degrees of removal with chemical treatment. Oil and grease were reduced 75 - 85% with acid, but only by 37% with alum. No significant reductions in cyanide, phenols or surfactants were measured. Ammonia, which occurs in the tailings pond at high levels, was reduced no more than 25% by any of the three treatments. With regard to nutrients, none of the treatments caused significant changes in dissolved organic carbon or total Kjeldahl nitrogen. Nitrification, as indicated by the reduction in ammonia and increase in nitrite and nitrate, appears to have been somewhat faster with alum. Phosphate was decreased somewhat with acid and increased with alum.

With regard to minor elements, treatments tended to have little effect on the relatively low concentrations present in tailings pond water. With both T2 and T3 there were increases in the concentrations of vanadium and zinc, and a decrease in the concentrations of barium and titanium. With acid treatment there was an increase in iron, manganese and molybdenum and a decrease in aluminum and antimony. Alum treatment increased aluminum and decreased arsenic and iron. However, these changes probably have little significance since the concentrations of all except boron are below the level deemed to be deleterious to aquatic biota (Train 1979).

b) Effect of storage on tailings pond water

The water in the surface zone of the tailings pond is affected by the continuous input of fresh tailings and the underlying sludge layer. Comparison of data in Table 1 with that obtained in 1980 (MacKinnon 1981) shows that there has been an increase in dissolved solids as well as a reduction in suspended solids. However, the toxicity has remained high, with LC_{50} to trout and Daphnia being less than 10% at all times. By placing tailings pond water in the 300 m^3 experimental pits, changes in the quality of pond water, free from the effects of fresh tailings input and out of contact with the sludge zone, could be followed.

Following filling, the volume of water in the two experimental pits was affected by evaporation, precipitation and freezing. These changes in water volume are reflected by changes in the concentration of chloride. After filling, chloride remained about 100 mg/L, but rose to 200 mg/L under the winter ice which was 0.7 to 1.0 m thick (Figure 3). Since dissolved solids are excluded during freezing, this indicates that about half of the water in the pits was frozen. During the spring, chlorine decreased to 70 - 80 mg/L as a result of dilution by melting snow and ice, and then increased as a result of evaporation. In September, 1984, chloride concentration was 110 mg/L. Seasonal changes in the concentrations of other major constituents (sulphate, total organic and inorganic carbon, sodium) were similar to that of chloride. Since the surface area of the pits (169 m^2) was large relative to the depth (3 m), mixing by wind prevented stratification except immediately after ice melt. Even under the ice differences between top and bottom of the water never exceeded 10%.

Concentration of dissolved oxygen in the surface zone of the tailings pond is less than 30% saturated. The continuous input of large quantities of fresh tailings (100×10^6 m^3/yr) with high concentrations of readily-oxidized material (i.e. organic carbon, phenols, ammonia, sulphide) keeps the C.O.D. at over 400 mg/L and the 5-day B.O.D. at over 30 mg/L (Table 1). However, once the tailings pond water was placed in the experimental pits, D.O. rose rapidly, reaching 100% saturation within 4 months (Figure 4). After freeze-up, D.O. decreased to 1 - 2 mg/L, indicating that the oxygen demand of the water was still very high. Samples collected in May, 1984, showed that the 10-months storage had decreased C.O.D. by 72% and B.O.D. by 94% (Table 1). Following ice-melt, D.O. again increased rapidly till the water was 100% saturated. Turbidity decreased rapidly at first but appears to have stabilized at 50 - 100 NTU after the first year of storage (Figure 5).

The values for Experimental Pit 1 given in Table 1 refer to water which was collected in May at a time when it was diluted about 30% by melt water (i.e. chloride was 69 mg/L compared to 100 mg/L on June 30, 1983). Hence the Pit 1 values should be increased about 1.4 times before comparing them with the other values in Table 1. With this correction, suspended solids would be about 216 mg/L, or over ten times higher than the level of reduction accomplished by

T2 in a matter of hours. Compared to T2, storage was not as effective in removing oil and grease, and like T2, did not result in any significant reduction in surfactants. However, unlike T2, storage removed most of the phenol and cyanide and some of the ammonia. Along with the reduction in ammonia there was was a signficant increase in nitrite and nitrate.

Toxicity of the tailings water, as measured with the Microtox Test, was reduced substantially during storage (Figure 6). After eight months, the EC_{50} had increased from 35% to 100% in both pits. Detoxification rate appeared to occur at about the same rate in winter as summer.

The reduction in acute toxicity was confirmed in bioassays with trout and Daphnia. As indicated in Table 1, the 96 h LC_{50} for both species was over 100% when tested with the water stored in the pits for ten months. The Daphnia and trout kept in full-strength water from Treatment T2 all survived (Table 2). When exposed to full strength tailings pond water which had been stored for ten months, 60% of the trout and 80% of the Daphnia survived. Taking into account that the water from the pits was about 30% diluted when tested as described above, one sees that the ten-month storage resulted in less detoxification than Treatment 2.

The 96 hour bioassay with trout were repeated in September, 1984, at which time the water in the pits was completely mixed and had been aged for 14 months. Whereas in Pit 1 the LC_{50} was greater than 100% and all fish survived in full-strength water, in Pit 2 the LC_{50} was 95% and only 20% of the trout survived in the full-strength water (Table 2). The reason for this variability is not clear.

c) Sequential testing procedure

As described above, we have used a variety of toxicity tests in studies on treatment procedures for wastewater resulting from the production of synthetic crude oil from oil sands. In general, we have used a hierarchical approach in which the results from one type of bioassay are used to determine if we should proceed with the next test in the sequence. We have found this sequential testing procedure extremely practical and feel that it has application in the development of treatment methods for other toxic wastewaters.

The four-stage testing procedure is built around the Beckman Microtox test, the 96-hour static trout bioassay and conventional biomonitoring of the receiving water (Figure 7). The first stage is an evaluation of the toxicity of the wastewater with the Microtox test. If the Microtox EC_{50} is 100% then one proceeds to the 96-hour trout bioassay. If EC_{50} is less than 100% one goes to Stage 2 where the Microtox is used to screen the effectiveness of various treatment methods. We chose this test for our routine work because it: [1] provided results in 15 minutes, [2] required only a 5 ml water sample, [3] used only a small bench area (75 cm x 150 cm), [4] cost little (about $3-5/test) and, [5] gave reproducible results with a fairly high degree of precision (±20%). As has been shown to be the case with other wastewaters, we have found that with tailings pond water the Microtox technique shows a good correlation with other tests (MacKinnon and Retallack 1982).

We subdivide Stage 3 depending on the parameter used in the test. Stage 3A is primarily concerned with the confirmation of the Microtox results with the trout bioassay. LC_{50} is used as the test parameter because if this value is less than 100% the wastewater is classified as unacceptable by regulatory agencies (Environment Canada 1974). It should be noted that an LC_{50} value of 100% indicates that 50% of the fish die in a full strength sample of the test solution during the 96-hour exposure period. In Stage 3B we go a step further and try to achieve 100% survival of trout in the full-strength test solution of tailings pond water. Currently, this level of detoxification of tailings pond water can be achieved with chemical treatment and we are close to achieving it with a 16-month storage period.

Once Stage 3B is achieved, further work would focus on design and cost engineering, obtaining approval from the required regulatory agencies, and construction of the treatment facility. During this time, a biomonitoring program would be developed as indicated in Stage 4. This involves surveys of the receiving water prior to discharge to establish baseline conditions both upstream and downstream of the proposed discharge point. Species abundance and diversity are the two most commonly used test parameters. Additional surveys would be conducted at regular intervals after initiation of discharge in order to assess the environmental impact of the effluent. Further information regarding the design of biomonitoring programs can be found in Hellawell (1978) and Green (1979).

ACKNOWLEGEMENTS

We like to thank Mr. Bill Benson and Mr. James Doyle, Syncrude Canada Ltd., for their expert technical assistance with sample collection and preparation and data analysis. Water analysis were done by Chemex Laboratories, Calgary, trout bioassays by Chemical and Geological Laboratories, Edmonton, and Daphnia bioassays by E.V.S. Consultants, Vancouver.

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Table 1. Comparison of physical, chemical and toxicological properties of water from the Athabasca River (AR) and Syncrude's Tailings Pond (TP), as well as that obtained by treatment of tailings pond water with acid (T1), with acid and flocculant (T2), with alum and flocculant (T3), and by 10-month storage in experimental pits (P1). Samples collected May 3, 1984. Numbers in brackets beside minor elements give detection limits in µg/L.

Parameter	Units	AR	TP	T1	T2	T3	P1
General Parameters							
pH	pH units	8.4	8.1	8.3	8.4	8.3	8.4
Conductivity	µS/cm	270	1410	1900	1790	1350	860
Dissolved solids	mg/L	134	1191	1750	1570	1300	659
Suspended solids	mg/L	55	1007	160	20	20	154
Turbidity	NTU	8	680	78	34	32	66
Color	TCU	30	1000	300	200	200	300
B.O.D.	mg/L	2	32	19	12	15	2
C.O.D.	mg/L	16	422	189	299	289	120
Tannin + Lignin	mg/L	0.6	9.0	4.5	3.5	3.5	1.8
Dissolved oxygen	mg/L	9.0	2.0	8.0	8.0	8.0	3.5
Major Ions							
Sodium	mg/L	13	395	540	525	390	232
Potassium	mg/L	1.5	9.0	8.5	9.1	8.5	5.2
Magnesium	mg/L	8.9	3.3	3.4	3.4	3.5	3.0
Calcium	mg/L	34.4	8.1	7.3	7.3	6.7	8.7
Chloride	mg/L	7	117	114	116	114	69
Sulphate	mg/L	26	215	605	595	250	158
Fluoride	mg/L	0.05	2.50	2.50	2.12	2.12	1.25
Total Inorganic C	mg/L	21	102	74	69	100	58
Alkalinity (as $CaCO_3$)	mg/L	154	542	344	322	508	264
Nutrients							
Total Organic C	mg/L	6.3	42.5	40.0	42.5	43.5	22.5
Total Kjeldahl N	mg/L	0.9	7.1	5.0	6.6	6.6	1.2
Nitrite + Nitrate-N	mg/L	0.02	0.02	0.04	0.08	0.12	0.86
Ammonia-N	mg/L	0.05	2.87	2.57	2.82	2.20	1.24
Phosphate-P	mg/L	0.003	0.070	0.030	0.020	0.200	0.041
Toxic Substances							
Oil & Grease	mg/L	0.9	19.0	5.0	2.9	12.0	5.4
Surfactants (MBAS)	mg/L	0.12	2.00	1.95	1.90	2.05	1.40
Phenols	mg/L	0.001	0.150	0.145	0.125	0.120	0.007
Cyanide	mg/L	0.001	0.950	0.920	0.920	0.950	0.013

Table 1 cont...

Parameter	Units	AR	TP	T1	T2	T3	P1
Minor Elements							
Aluminum (10)	µg/L	10	140	130	90	160	--
Antimony (0.2)	µg/L	2.6	12.0	12.0	6.5	12.0	--
Arsenic (0.1)	µg/L	1.0	7.1	9.4	10.5	4.7	6.7
Barium (10)	µg/L	250	230	180	130	90	--
Boron (20)	µg/L	60	1680	1740	1720	1720	890
Cadmium (1)	µg/L	<1	<1	<1	<1	<1	<1
Chromium (1)	µg/L	<1	4	1	<1	<1	--
Cobalt (1)	µg/L	<1	<1	3	<1	<1	2
Cooper (1)	µg/L	1	2	<1	<1	<1	1
Iron (10)	µg/L	110	390	510	760	10	870
Lead (2)	µg/L	<2	<2	<2	<2	<2	<2
Manganese (4)	µg/L	15	41	95	57	44	107
Mercury (0.10)	µg/L	<0.10	0.16	<0.10	0.10	0.10	0.25
Molybdenum (1)	µg/L	--	63	110	110	62	--
Nickel (1)	µg/L	<1	14	15	14	15	9
Selenium (0.2)	µg/L	<0.2	10.6	9.5	12.2	9.7	--
Silica (as SiO_2)(20)	µg/L	3600	4500	5250	4520	4500	3350
Silver (1)	µg/L	<1	<1	<1	<1	<1	--
Titanium (10)	µg/L	50	60	<10	<10	<10	<10
Vanadium (1)	µg/L	10	24	100	136	34	183
Zinc (1)	µg/L	4	1	19	19	8	2
Toxicity							
Microtox	LC_{50} (%)	>100	43	>100	>100	45	>100
Trout	LC_{50} (%)	>100	7	>100	>100	7	>100
Daphnia	LC_{50} (%)	>100	2	93	>100	38	>100

Table 2. Summary of 96-hour static trout bioassays with tailings pond water from various sources and stored for various times. Numbers in parentheses indicate fish showing stress. The recycle pond holds tailings pond water prior to its recycling to the extraction plant. Pits 3 and 4 are identical to Pits 1 and 2.

Source of water	Storage time	No. fish surviving in various dilutions and control water							LC_{50}
		Control	5%	10%	20%	50%	75%	100%	
1) Tailings Pond May, 1984	0 days	10	10(4)	0	0	-	-	-	7%
2) Tailings Pond Sept., 1984	0 days	5	5	1(1)	0	-	-	-	8%
3) Recycle Pond Sept., 1984	1-2 days	5	5	5(2)	0	-	-	-	15%
4) Pit 3 Sept., 1984	5 mos.	5	-	5	5	5	0	0	60%
5) Pit 4 Sept., 1984	5 mos.	5	-	5	5	5	0	0	60%
6) Pit 1 May, 1984	10 mos.	10	-	10	10	10	10(1)	6(6)	>100%
7) Pit 1 Sept., 1984	14 mos.	5	-	5	5	5	5	5	>100%
8) Pit 2 Sept., 1984	14 mos.	5	-	5	5	5	5	1(1)	90%
9) Acid Treatment	1 day	10	-	10	10	10	10	10	>100%
10) Acid & Floc Treatment	1 day	10	-	10	10	10	10	10	>100%
11) Alum Treatment	1 day	10	10(4)	0	0	--	--	--	7%

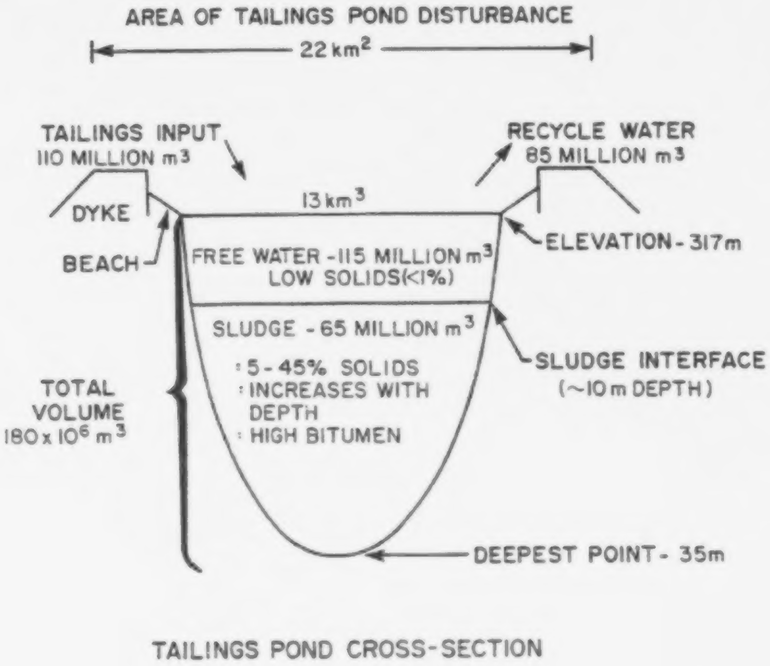
AREA OF TAILINGS POND DISTURBANCE
22 km²
TAILINGS INPUT
110 MILLION m3
RECYCLE WATER
85 MILLION m3
DYKE
13 km³
BEACH
FREE WATER - 115 MILLION m³
LOW SOLIDS(<1%)
ELEVATION - 317m
SLUDGE - 65 MILLION m³
: 5 - 45% SOLIDS
: INCREASES WITH DEPTH
: HIGH BITUMEN
SLUDGE INTERFACE
(~10 m DEPTH)
TOTAL VOLUME
180 x 10⁶ m³
DEEPEST POINT - 35m
TAILINGS POND CROSS-SECTION

Figure 1. Schematic cross-section of Syncrude Canada Ltd. tailings pond, July 1984. Volume of tailings input excludes sand.

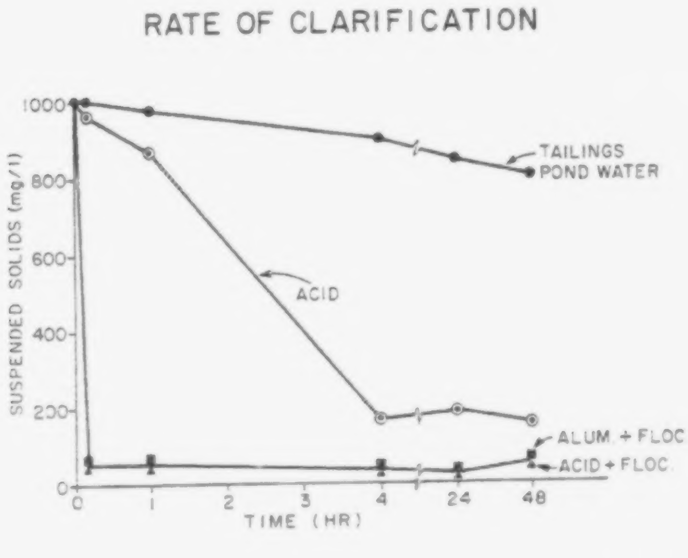

Figure 2. Effect of three treatment methods (acid alone, acid and flocculant CFA30, alum and flocculant) on the rate of clarification of tailings pond water.

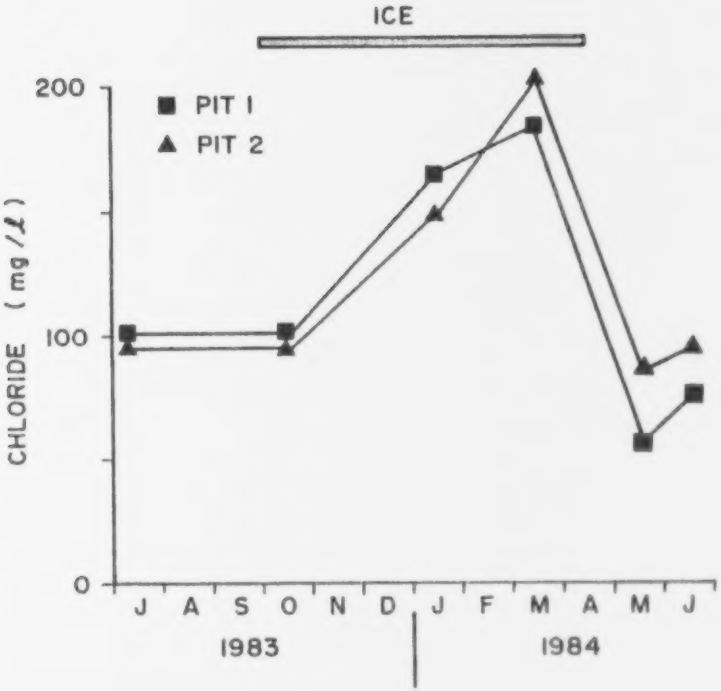

Figure 3. Seasonal changes in the concentration of chloride in experimental pits 1 and 2. Data based on samples collected 25cm below the water surface.

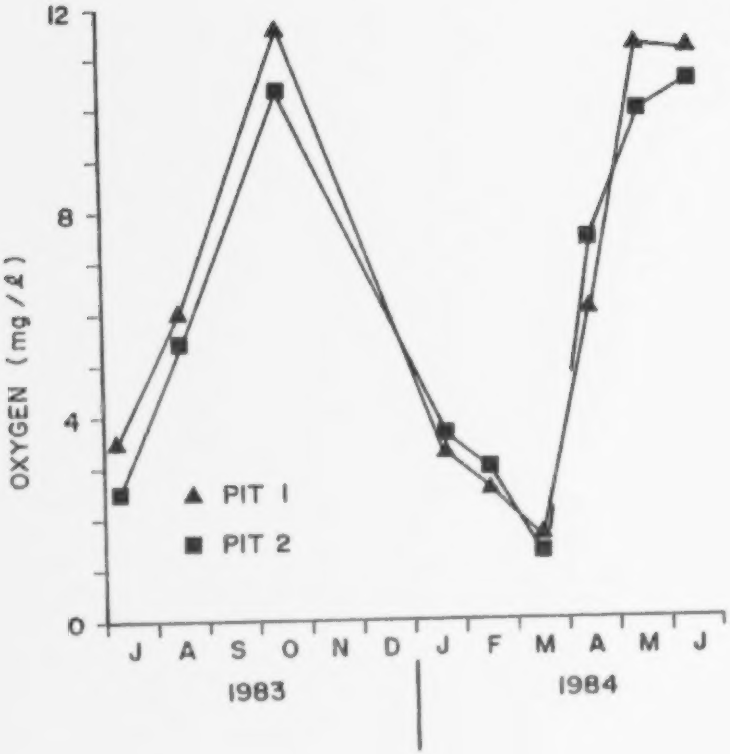

Figure 4. Seasonal changes in the concentration of dissolved oxygen in experimental pits 1 and 2. Data based on samples collected 25cm below the water surface.

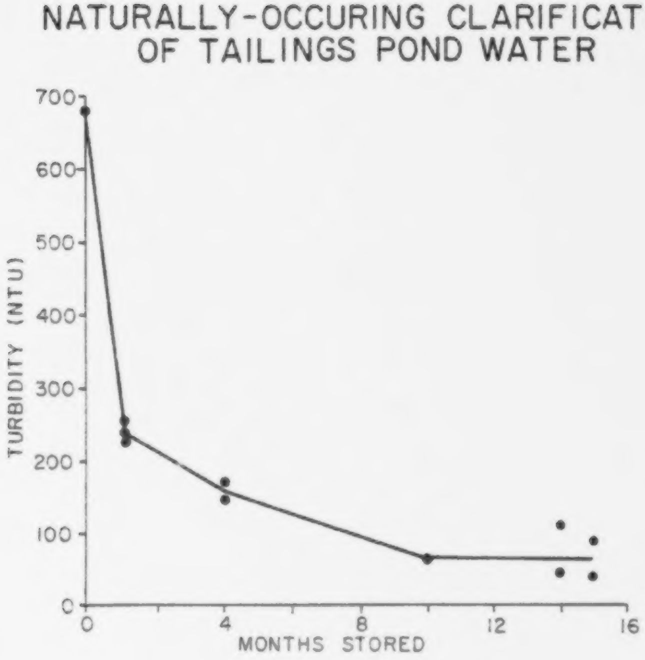

Figure 5. Changes in turbidity of tailings pond water during storage in experimental pits.

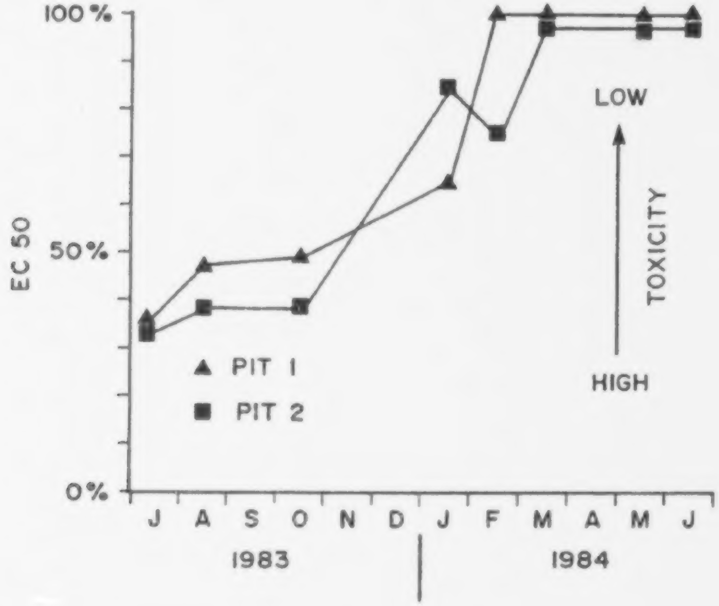

Figure 6. Changes in toxicity of tailings pond water placed in experimental pits 1 and 2.

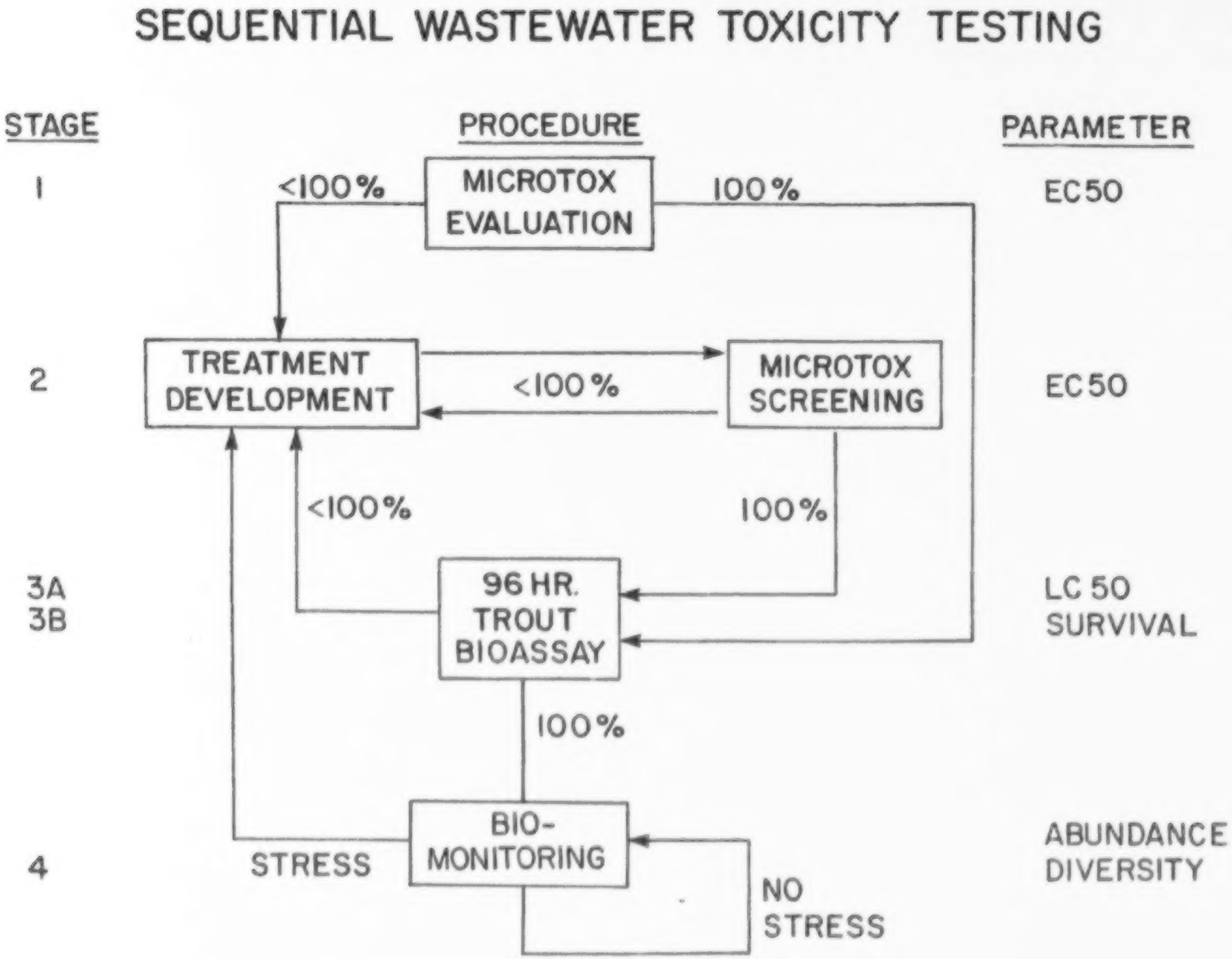
SEQUENTIAL WASTEWATER TOXICITY TESTING
STAGE
PROCEDURE
PARAMETER
1
<100%
MICROTOX EVALUATION
100%
EC50
2
TREATMENT DEVELOPMENT
<100%
MICROTOX SCREENING
EC50
<100%
100%
3A
3B
96 HR. TROUT BIOASSAY
LC 50
SURVIVAL
100%
4
STRESS
BIO-MONITORING
NO STRESS
ABUNDANCE
DIVERSITY

Figure 7. Schematic representation of the sequential toxicity testing procedure for wastewaters. The right-hand column lists the parameters measured at each stage. See text for details.

ACUTE LETHALITY OF OIL BASED DRILLING FLUIDS AND COMPONENTS

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In 1982 the Environmental Protection Service (EPS), Environment Canada became aware of plans to use new "low toxicity" mineral oil based drilling muds in Canadian offshore exploration programs. Recent concern about the adverse environmental effects of discharging diesel and other high aromatic content oil based drilling muds and cuttings into the ocean prompted the industry to investigate the use of potentially less toxic oils in their drilling fluids. In an effort to document the toxicity of the new drilling muds and component chemicals EPS Atlantic undertook screening toxicity tests in cooperation with the drilling fluid supply companies. Toxicity tests were static 96 h LC_{50}s using the three-spined stickle-back (Gasterosteus aculeatus). Initial testing was conducted on laboratory prepared drilling muds, formulated by the companies to EPS specifications. This was followed by testing of a used mud formulation from Texas. The LC_{50}s of the prepared mineral oil base drilling muds ranged from 68,000 ppm to >320,000 ppm while the LC_{50} of the used mud was greater than 58,000 ppm. In comparison, water based drilling muds has LC_{50}s ranging from <10,000 ppm to 100,000 ppm and diesel and other high aromatic content drilling muds had LC_{50}s of 2600 ppm and <1,000 ppm. Individual component chemicals of the prepared fluids were also tested. LC_{50}s ranged from 6.5 ppm to >100,000 ppm. Analysis of the data suggests that the acute toxicity of the "low toxicity" drilling muds were due mainly to the emulsifiers and lime.

LÉTALITÉ AIGUË CAUSÉE PAR LES BOUES DE FORAGE ET COMPOSÉS À BASE DE PÉTROLE

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En 1982, Le Service de Protection Environnementale (SPE), Environnement Canada, fut mis au courant des plans en vue d'utiliser les nouvelles boues de forage à base d'huile minérale à "basse toxicité" dans les programmes d'exploration au large des côtes du Canada. De récentes inquiétudes à propos des effets négatifs sur l'environnement causés par les décharges d'huile diesel, d'autres boues de forage à base d'huile à haut contenu aromatique et de déchets dans l'océan ont forcé les industries à examiner l'utilisation d'huiles potentiellement moins toxiques dans leurs liquides de forage. Dans un effort pour documenter la toxicité des nouvelles boues de forage et de composés chimiques, SPE Atlantique a entrepris des analyses de dépistage de toxicité en coopération avec les compagnies productrices de liquides de forage. Les analyses de toxicité étaient des CL_{50}s statiques de 96 heures utilisant des épinoches à trois épisodes (Gasterosteus aculeatus). Les analyses initiales furent conduites en laboratoire sur des boues de forage préparées selon les spécifications de la SPE. Cette étude fut suivie par l'analyse d'une boue usée dont la formulation provient du Texas. Les CL_{50}s des boues de forage à base d'huile minérale préparée variaient de 68,000 ppm à plus de 320,000 ppm, alors que le CL_{50} de la boue usée était de plus de 58,000 ppm. En comparaison, les boues de forage à base d'eau avaient des CL_{50}s variant de <10,000 ppm à 100,000 ppm, et l'huile diesel et autres boues de forage à haut contenu aromatique avaient des CL_{50}s de 2,600 ppm et de <1,000 ppm. Des composés chimiques individuels provenant de fluides préparés furent aussi analysés. Les CL_{50}s variaient de 6.5 ppm à >100,000 ppm. L'analyse de ces donées suggère que la toxicité aiguë des boues de forage à "basse toxicité" est principalement due aux émulsifiants et à la chaux.

INTRODUCTION

Traditionally, oil based drilling muds even at low concentrations have been considered acutely lethal to fish and other aquatic organisms. This assumption has been based on the fact that, until recently, oil based drilling muds were formulated using diesel oil, crude oil or various high aromatic content distillates of crude oil. This type of drilling mud has now been used throughout the world and has continually raised concerns about its potential to adversely affect the marine environment. These concerns led to the development of an alternate oil based drilling mud with significantly lower acute lethality to marine organisms. The new base oils are clear low aromatic mineral oils. The lower toxicity is generally attributed to the reduced aromatic content and the lower polynuclear aromatic hydrocarbon (PAH) content.

In 1982, after learning that oil based drilling mud was being considered for use in the Canadian offshore oil exploration programs, the Environmental Protection Service (EPS), Atlantic Region began to gather data on the acute toxicity to marine organisms of both diesel oil and the new mineral oil based drilling muds. Although there were some data available, most pertained to marine biota from the North Sea and Gulf of Mexico and there was very little data on species endemic to the Canadian marine environment. This lack of data and other uncertainties associated with the release of oil based drilling muds to the marine environment prompted EPS Atlantic to conduct preliminary toxicity screening of the whole muds, component chemicals and base oils using an east coast organism.

Drilling muds, whether oil based or water based, perform essentially the same function. The mud is pumped down the drill pipe out through nozzles in the bit, and then up along the drilled hole to the surface (Fig. 1). The mud functions to balance down-hole pressure, cool and lubricate the drill pipe and bit, remove drill solids (cuttings) from the hole and form a filter cake on the walls of the drilled hole to reduce loss of fluid to the formation or influx of fluids to the drilled hole. When the circulated mud is returned to the surface it is passed through a processing unit (shale shaker) to remove the cuttings and recover the mud. The cuttings are then discharged overboard and the mud is reused. Normally, the discharged cuttings will sink to the bottom within a few hundred meters of the oil drilling rig. However, currents in the area can significantly effect the distribution of cuttings on the sea floor.

METHODS AND MATERIALS

The Environmental Protection Service prepared a list of specifications for the preparation of the laboratory prepared drilling muds (Table 1). This allowed the direct comparison of various company products without having to consider differences in mud properties. The weight of the mud formulation (14 lbs/gal) was considered an average for deep sections of wells drilled on the east coast and the 90:10 oil water ratio a likely maximum for both oil and additives for this formulation.

Many of the materials tested in this study are considered proprietary chemicals and to permit distribution of the comparative toxicity data it was necessary to keep the trade names of the muds and their components confidential. Hence the laboratory prepared mineral oil muds are labelled A to I; used mineral oil mud is K, and the laboratory prepared diesel oil mud is J and the used diesel oil mud L. The components are identified as oil (A) meaning base oil for mud A, or emulsifier (I, J) meaning an emulsifier present in muds I and J, etc. Because several of the muds were formulated by the same company some components may be used in more than one mud. There is also some uncertainty as to whether all component chemicals were identified by the companies and at least two samples were tested without knowing their component chemicals.

The tests were conducted at EPS laboratories in Nova Scotia (N.S.) and Newfoundland (Nfld.). All tests were static, aerated, 20 or 40 litre acute lethal toxicity tests of 96 hours duration. Aeration rate was 200 ml/min. The test fish used were the three-spine stickleback, (Gastrosteus aculeatus) seined at Lawrencetown, N.S. and Bellevue Beach, Nfld.

Fish were held in fiberglass tanks with continuously flowing aerated sea water. The fish were fed either Nutrafin® fish food (N.S.) or blended herring and liver (Nfld.) at a rate of approximately 3% of the total body weight per day. The fish were not fed for 24 hours prior to the start of the tests or during the toxicity tests. Fish weight ranged from 0.3 to 1.0 g (N.S.) and from 0.8 g to 2.0 g (Nfld.). Loading densities ranged from 1 to 4 litres of test solution per gram of fish per day (N.S.) and from 0.5 to 1.3 litres of test solution per gram of fish per day (Nfld.). Test solutions were measured daily for temperature, dissolved oxygen and pH. All tests were conducted at 15±2°C. The salinities ranged from 29 to 31o/oo for filtered Bedford Basin seawater used in Nova Scotia and from 28o/oo to 32o/oo for unfiltered sea water used in Newfoundland. A test was repeated if there was greater than 20% control mortality.

The test method used was that of Wong (1982). This method requires an initial pH adjustment of the test solution to within 0.5 pH units of the control water. In this study, where necessary, 1N HCI was used to lower the pH. Test materials were either stirred directly into the dilution water using a clean plastic rod or blended with water from the test tank using a blender then added to the test tank and stirred. Because of the often highly-turbid nature of the test solutions, the test fish were kept in cages made of nontoxic Vexar (high density polyethylene) with mesh size 4.8 mm in order to facilitate fish checks.

Calculation of test results were undertaken according to the method of Litchfield and Wilcoxon (1949) and test results were expressed as 96-hour LC_{50} values. Because no partial mortalities occurred in the tests, confidence limits could not be calculated. The ranges of toxicity values, (concentration nearest the LC_{50} value causing 0% and 100% mortality) are provided.

RESULTS AND DISCUSSION

The acute lethal toxicity of the oil based drilling muds and their components is given in Tables 2, 3, 4 and 5. All values are expressed as mg/L, nominal concentrations. The 96 hour LC_{50}s for the muds ranged from <1,000 mg/L to 320,000 mg/L. Often, the LC_{50} values decreased after 10 days exposure (muds C, D and H) but remained at 320,000 mg/L for muds E and G. Several muds (A, C, and D) caused the pH of the test solutions to rise throughout the tests, in spite of the initial pH neutralization, and undoubtedly contributed to the overall acute lethality.

The acute lethality of the oils, emulsifiers, and other component chemicals are presented in Tables 3, 4 and 5, respectively. The mineral oils were all found to be virtually non-toxic with 96 h LC_{50}s ranging from >10,000 mg/L to >18,000 mg/L. Test fish were exposed to oils E, F, and G for 10 days with no increase in mortality. This is in general agreement with studies reported previously on the low acute lethality of the mineral oils (e.g., Anonymous, 1982; Franklin, 1982; Thoresen and Hinds, 1983; Blackman, et al. 1982). However, long term effects may be possible. The LC_{50}s for emulsifiers ranged from 27 mg/L for emulsifier #3 (A) to >100,000 for emulsifier (I, J) (Table 3). The more water miscible products tended to be more toxic (K. Doe and J. Osborne, personal observations). The LC_{50}s of other components ranged from 6.5 mg/L for the oil wetting agent and thinner (I, J) to >56,000 mg/L for the fluid loss control agent (I, J) (Table 4). The weighting agent barite, a major component of many drilling muds, was non-acutely lethal up to a concentration of 32,000 mg/L. This is in good agreement with the reported low acute lethality for barite (Sprague and Logan, 1979). The lime, which can raise the pH of the test solutions, had an LC_{50} of 170 mg/L when not neutralized but an LC_{50} of >2,000 mg/L when neutralized.

It is obvious from the data that the mineral oil muds were considerably less toxic (LC_{50} 6.5% to <32%) than the diesel oil muds (LC_{50}<1,000 and 2,600 ppm). It should be noted, however, that the diesel muds in general have been found to be more acutely lethal than mineral oil based drilling muds (Hinds et al., 1983). It can be seen that diesel oil had a higher acute lethality (lower LC_{50}) than any of the mineral oils (Table 3). This could lead to the higher acute lethality of diesel oil based muds.

Identification of the toxic components was attempted for several muds (muds A, E, F, G and H). It was possible to calculate the theoretical contribution of each component (in toxic units*) to the toxicity of the whole mud. For example, in mud A (Table 6), one toxic unit of mud theoretically contained 30 toxic units of emulsifier #3 (A) and 4 toxic units of lime. Other components do not appear to contribute greatly to the toxicity of the mud. It appears that the toxicity of the whole mud is much less than would be predicted from additive toxicity of its components. This may result from lowering the pH of the test solutions, coupled with the possibility that the emulsifier #3 (A) might be made less available to the test organisms either by the mineral oil, the barite or other particulates present in the whole mud. It thus appears that by lowering the toxicity of emulsifier (A), the mud could possibly be made less toxic. Similar analyses for muds E, F, G and H suggest that emulsifier #2 (E, F, G, H) would be the component of concern from an acute lethality viewpoint.

It is important to note that all the drilling muds, except K and L, tested in this study were "laboratory" muds. Muds K and L were used mud from two active well sites. Other components which might be present in the "field" muds, such as biocides used to inhibit bacterial decomposition of organic materials in the muds and/or hydrocarbons picked up from the formations being drilled, may

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*Number toxic units =

$$\frac{\text{Concentration of the material (mg/L)}}{\text{96 h } LC_{50} \text{ of the material (mg/L)}}$$

increase toxicity over that present in "laboratory" muds. In addition, high temperatures and pressures encountered downhole could alter the toxicity of oil based drilling muds.

The results of the present study provide screening data for comparison of the acute lethality of twelve oil based drilling muds and some of their components. Several muds tested had LC_{50} values four or more times higher than mud A (i.e., less acutely lethal than mud A) and when compared with two water based drilling muds tested by EPS using the same test methodology, the majority of mineral oil based drilling muds were less acutely lethal to three-spine stickleback than these water-based muds which had 96 h LC_{50}s of <10,000 mg/L and 100,000 mg/L. It is possible that oil based drilling muds, because of their oil content, could cause anaerobic conditions in heavily contaminated sediments (Groenewold et al., 1982). In addition, the availability of hydrocarbons from oil based drilling muds to aquatic organisms has been sparsely studied.

CONCLUSIONS

[1] The 96 h LC_{50}s for the mineral oil based drilling muds ranged from 68,000 mg/L for mud A to >320,000 mg/L for mud E, G, H and I. The diesel oil mud J had a 96 h LC_{50} of <1,000 mg/L, the used mineral oil mud had an LC_{50} of <58,000 ppm and the used diesel mud had an LC_{50} of 2,600 mg/L.

[2] The lethality of the whole muds A, C and D was partially due to the high pH of the test solutions. Certain toxic emulsifiers may also have contributed to the acute lethality of the various whole muds.

[3] The 96 h LC_{50}s for the mineral oils ranged from >10,000 mg/L to >18,000 mg/L. Three samples of diesel oil, tested independently of this study, had LC_{50} values of >320 mg/L.

[4] The 96 h LC_{50}s of the emulsifiers tested ranged from 27 mg/L to 100,000 mg/L.

[5] Of the miscellaneous components, oil wetting agent and thinner (I, J) had the highest toxicity (96 h LC_{50} of 6.5 mg/L). Others were virtually non-toxic e.g. viscosifiers for mud B, E, F, G and H; barite; fluid loss control agent (I, J); and filtration control agent (E, F, G, H). Lime was found to be toxic (96 h LC_{50} 170 mg/L) but its toxicity was greatly reduced if the pH was neutralized (96 h LC_{50} >2,000 mg/L).

[6] The majority of mineral oil based drilling muds were less toxic than "field" samples of water based drilling muds tested previously.

[7] Long term studies on the availability and chronic effects of hydrocarbons from oil based drilling muds on benthic organisms (such as clams, polychaetes and sea urchins) would be useful and are recommended.

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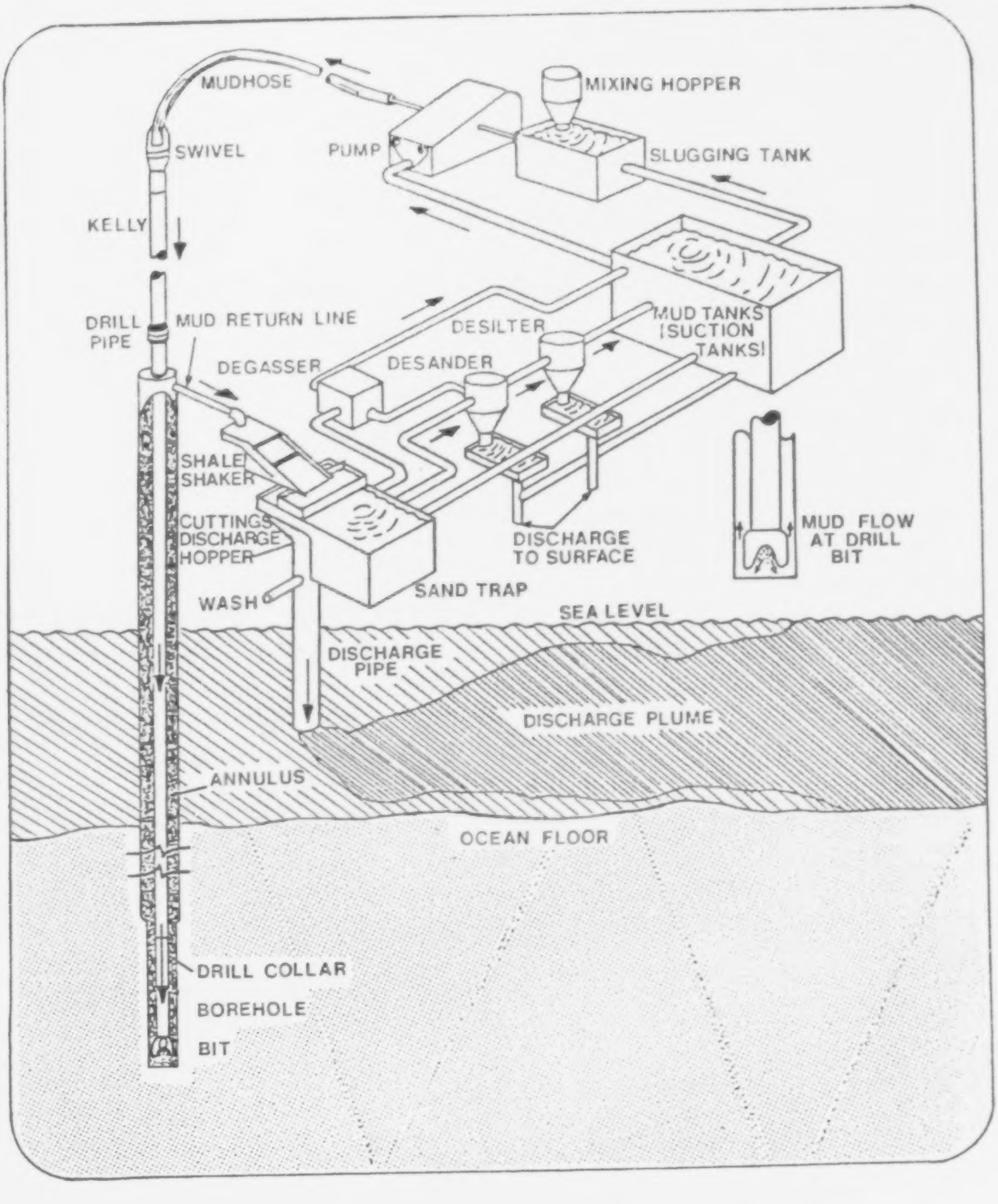
MUDHOSE
MIXING HOPPER
SWIVEL
PUMP
SLUGGING TANK
KELLY
DRILL PIPE
MUD RETURN LINE
DESILTER
MUD TANKS (SUCTION TANKS)
DEGASSER
DESANDER
SHALE SHAKER
CUTTINGS DISCHARGE HOPPER
DISCHARGE TO SURFACE
MUD FLOW AT DRILL BIT
WASH
SAND TRAP
SEA LEVEL
DISCHARGE PIPE
DISCHARGE PLUME
ANNULUS
OCEAN FLOOR
DRILL COLLAR
BOREHOLE
BIT

Figure 1. Drilling mud system.

Table 1. Properties for laboratory prepared drilling muds.

Formulation:

- mud weight (PPG)	14.0
- oil water ratio	90:10
- $CaCl_2$ percent by weight	30

Performance Data:

Plastic viscosity	34 at 115°F
Yield Point	11 at 115°F
Gels	6/7
Electrical stability	900 volts
API fluid loss (30 min)	5.8 cc
HPHT fluid loss (300/500)	23 cc

Table 2. Acute lethal toxicity of oil-based drilling muds to the three-spine stickleback *Gasterosteus aculeatus* in sea water. LC_{50} values expressed as mg/L, nominal concentrations. All muds stirred into dilution water.

Test Mud	96 h LC_{50}		10 day LC_{50}	
A	68,000	(R*: 18,000 - 180,000)	-	
B	135,000	(R: 100,000 - 180,000)	-	
C	260,000		225,000	(R: 180,000-270,000)
D	>250,000		<180,000	
E	>320,000		>320,000	
F	290,000		290,000	
G	>320,000		>320,000	
H	>320,000		< 56,000	
I	>320,000		-	
J	< 1,000		-	
K	> 56,000		> 5,600	
L	2,600			

*R = Closest range of 0 and 100% mortality to the LC_{50} value.

Table 3. Acute lethal toxicity of the mineral oils used in the various oil-based drilling muds and diesel oil to the three-spine stickleback *Gasterosteus aculeatus* in seawater. LC_{50} values expressed as mg/L, nominal concentrations.

Test Material	96 h LC_{50}	10-day LC_{50}
Mineral oil (A) - Stirred	>10,000	-
Mineral oil (A) - Blended	>10,000	-
Mineral oil (B) - Stirred	>10,000	-
Mineral oil (B) - Blended	>10,000	-
Mineral oil (E) - Blended	>10,000	>10,000
Mineral oil (F) - Blended	>10,000	>10,000
Mineral oil (G) - Blended	>10,000	>10,000
Mineral oil (H) - Blended	>10,000	-
Mineral oil (I) - Blended	>18,000	-
Diesel oil - Sample #1	> 320	
Diesel oil - Sample #2	430	
Diesel oil - Sample #3	460	

Table 4. Acute lethal toxicity of the emulsifiers used in the various oil-based drilling muds to the three spine stickleback *Gasterosteus aculeatus* in seawater. LC_{50} values expressed as mg/L nominal concentrations. All emulsifiers blended into dilution water.

Test Material	96-hour LC_{50}
Emulsifier #1 (A)	5,600 (R*: 3,200 - 10,000)
Emulsifier #2 (A)	2,700 (R: 1,000 - 10,000)
Emulsifier #3 (A)	27 (R: 10 - 100)
Emulsifier #1 (B)	32
Emulsifier #2 (B)	> 10,000
Emulsifier #1 (E,F,G,H)	7,500 (R: 5,600 - 10,000)
Emulsifier #2	32.9 (95% CL**: 21.6 - 44.1)
Emulsifier (I)	> 1,000 (40% mortality at 1,000 mg/L)
Emulsifier (I,J)	>100,000
Emulsifier (J)	> 5,600

*R = Closest range of 0 and 100% mortality to the LC_{50} value.

**95% CL = 95% Confidence Limits.

Table 5. Acute lethal toxicity of miscellaneous chemicals used in the various oil-based drilling muds to the three-spine stickleback *Gasterosteus aculeatus* in seawater. LC_{50} values expressed as mg/L nominal concentrations.

Test Material	96-hour LC_{50}
Fluid loss control agent (A) - Stirred	>32,000
Gelling agent (A) - Stirred	>32,000
Wetting agent (A) - Stirred	80
Viscosifier (B) - Stirred	>32,000
Weighting agent (barite) B - Stirred	>32,000
Component of Mud B - function not stated - Stirred	>32,000
Viscosifier (E,F,G,H) - Stirred	>32,000
Filtration control agent (E,F,G,H) - Stirred	>32,000 (40% mortality at 32,000)
Lime-pH not neutralized (E,F,G,H) - Stirred	170 (R*:56-180)
Lime-pH neutralized (E,F,G,H) - Stirred	> 2,000
Oil wetting agent and thinner (I,J) - Blended	6.5 (R:5.6-7.5)
Fluid loss control agent (I,J) - Blended	>56,000
Calcium chloride (Anhydrous) - Stirred	6,800 (R:1,000-30,000)

*R = Closest range of 0 and 100% mortality to the LC_{50} value.

Table 6. Approximate composition of mud A and approximate contribution of its components to the toxicity of the whole mud.

Component	Amount in Whole Mud	Amount as A % of Whole Mud (wt/wt)	Toxic Units of Individual Components in 1 Toxic Unit of Whole Mud
Oil P	0.6498 barrels	32.56	Not calculable, but <2.2
Emul #2	2 pounds/barrel	0.338	0.09
Emul #3	7 pounds/barrel	1.18	29.7
Lime*	4 pounds/barrel	0.675	4.3
Gel-A	10.5 pounds/barrel	1.77	Not calculable, but <0.04
H_2O	0.0709 barrels	4.19	Non-toxic
$CaCl_2$ (96%)	11.3 pounds/barrel	1.91	0.19
Barite	339.9 pounds/barrel	57.38	Not calculable, but <1.2

*Toxic unit concentration based on a 96 h LC_{50} to three-spine stickleback of 107 mg/L (pH not neutralized) for a lime sample received from another OBDM manufacturer.

COMPARATIVE SOLUBILITY AND ACUTE TOXICITY TO *DAPHNIA MAGNA* OF COAL LIQUIDS, SHALE OIL AND PETROLEUM

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Evaluating toxicity of complex organic mixtures to aquatic biota involves generating and characterizing water-soluble fractions (WSF) of test materials. Depending on the mixture, WSFs may contain several biologically active compound classes including phenolics, aromatic and saturate hydrocarbons, aromatic amines, sulfur heterocycles, and others. Although some components (e.g. phenolics) may predominate, each contributes to overall toxicity. We studied the relationships among solubility, chemical composition, toxicity, and acute hazard to *Daphnia magna* of several fossil-derived materials. These included coal liquids with different boiling ranges, coal liquids produced by different technological processes and under different process conditions, coal liquids derived from different source coals, a shale oil, and crude and refined petroleum.

Results indicated that concentrations of water-soluble components varied with component solubility and chemical composition of parent material, provided that mixing conditions were similar. The acute hazard to *D. magna* reflected both inherent toxicity and solubility of chemical components but, in most cases, could be predicted from concentrations of total carbon in solution. Coal liquids were generally more soluble in water than shale oil and petroleum materials and, thus, posed a greater potential toxic hazard to aquatic biota. Lower-boiling-range coal liquids were most soluble and, thus, posed the greatest acute hazard in water. Coal liquids derived from different coals had different solubilities and therefore showed minor differences in their potential acute hazard to *D. magna*.

SOLUBILITÉ RELATIVE ET TOXICITÉ AIGUË DE LIQUIDES DE HOUILLE, HUILE DE SCHISTE ET PÉTROLE ENVERS DAPHNIA MAGNA

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L'évaluation de la toxicité de mélanges organiques complexes sur le milieu aquatique implique la production et la caractérisation de fractions hydrosolubles (FHS) des matériaux analysés. Selon le mélange, les FHSs peuvent contenir plusieurs composés biologiquement actifs comprenant des phénols, des hydrocarbures aromatiques et saturés, des amines aromatiques, des hétérocycles sulfuriques, etc. Bien que quelques composés (e.g. phénols) puissent être prédominants, chacun contribue à la toxicité. Nous avons étudié les relations entre la solubilité, la composition chimique, la toxicité et le danger que plusieurs matériaux dérivés de fossiles posent à Daphnia magna. Ces matériaux comprenaient des liquides de houille avec différents points d'ébullition, des liquides de houille produits par différents procédés technologiques et sous différentes conditions, des liquides de houille dérivés de différentes sources de charbon, de l'huile de schiste et du pétrole brut et raffiné.

Les résultats indiquent que les concentrations de composés hydrosolubles varient avec la solubilité de composés et avec la composition chimique du matériel d'origine, en autant que les conditions de mélange soient similaires. Le danger posé à D. magna provient de la toxicité inhérente et de la solubilité des composés chimiques mais peut être prédit dans la plupart des cas par la concentration totale de carbone en solution. Les liquides de houille sont généralement plus solubles dans l'eau que l'huile de schiste et que les matériaux à base de pétrole et posent par conséquent un plus grand danger potentiel de toxicité au milieu aquatique. Les liquides de houille à bas point d'ébullition étaient les plus solubles, et posaient donc un plus grand danger dans l'eau. Les liquides de houille dérivés à partir de différents gisements avaient des solubilités différentes et ne montraient par conséquent que des différences mineures dans le danger potentiel posé à D. magna.

INTRODUCTION

Engineering options to produce synthetic fuels (coal liquids and shale oils) have been under development in the United States for more than a decade. The ecological implications of spilling these materials during transportation have been under investigation for several years (Gray and Druker 1981; Strand and Vaughan 1981; Mahlum et al. 1981; Gray and Cowser 1982; Gray 1984). Water-soluble fractions (WSFs) derived from coal liquids are of particular concern because of their known toxicity to various aquatic species (Bean et al. 1981; Dauble et al. 1982, 1983a,b; Gray et al. 1982; Becker et al. 1983; and others). Because of their chemical composition and solubility characteristics, coal liquids pose a greater hazard to aquatic biota than do fuel oils presently in commerce (Giddings et al. 1980; Giddings and Washington 1981; Gray et al. 1982; States et al. 1981; Ullrich and Millemann 1983).

Organically complex WSFs may contain several biologically active compound classes, including phenolics, aromatic and saturate hydrocarbons, aromatic amines, sulfur heterocycles, and others. Toxicological properties of WSFs from various coal-derived liquids largely reflect the presence of highly soluble phenolic constituents (Dauble et al. 1983a,b), whereas the WSFs of petroleum contain primarily aromatic hydrocarbons (Clark and Brown 1977). However, while some components (e.g. phenolics) may predominate in coal liquids, each contributes to overall toxicity. Engineering strategies that reduce concentrations of toxic water-soluble components of energy-related materials are important for technology development. Identification and characterization of bioactive agents in parent materials provide information toward this end. Therefore, we studied the relationships among solubility, chemical composition, and acute toxicity to *Daphnia magna* of several fossil-derived materials.

MATERIALS AND METHODS

Experimental materials included coal liquids with different boiling ranges, coal liquids produced by different processes (Solvent Refined Coal [SRC]-II, EDS, Integrated Two-Stage Liquefaction [ITSL]) and under different process conditions (EDS bottoms recycle versus once-through), and coal liquids (EDS recycle solvent) derived from different source coals (Illinois No. 6, Wyodak, Texas lignite). Toxicities of WSFs of these materials were compared with those of WSFs derived from shale oil (Oxidental), crude petroleum (Prudhoe Bay), and refined oils (No. 2 and No. 6 fuel oils).

Coal liquids were obtained from pilot plants and a process development unit (PDU). The SRC-II liquid (2.9:1 blend of middle to heavy distillates) and EDS process solvents were from pilot plants in Fort Lewis, Washington (operated by the Pittsburg & Midway Coal Mining Co.) and Baytown, Texas (operated by Exxon Research and Engineering Co.) respectively. The ITSL material was obtained from a PDU (operated by the C.E. Lummus Co.) in New Brunswick, New Jersey. Shale oil was obtained from Occidental Oil Shale, Inc. and was designated "heater treater - Retort #6". Petroleum materials were obtained from distributors in the Pacific Northwest and contained no preservatives.

WSFs were generated from parent materials using methods previously described by Dauble et al. (1983b). Test solutions to which *D. magna* were exposed were based on percent dilution of the WSF and on total carbon (TC) concentrations in the WSF. Parent materials were stored in the dark at 4°C, and new WSFs were generated for replicate tests. The experimental design was desribed in Dauble et al. (1983b). Because some bioassays were of a screening nature, 48 h LC_{50}s were determined by the graphical method (APHA 1976). For selected materials, LC_{50}s were determined by probit analysis (Finney 1971), and 95% confidence intervals were estimated to compare acute toxicity. Toxicity thresholds were expressed as percent WSF and mg/L TC based on dilution. The threshold (LC_{50}) expressed as percent WSF reflects both solubility and inherent toxicity of WSF components and therefore is an indication of the potential hazard to aquatic populations (see States et al. 1981). The threshold expressed as mg/L TC reflects only inherent toxicity of carbon-containing components in solution.

RESULTS AND DISCUSSIONS

Previous studies with several coal liquids (Dauble et al. 1983b) indicated that typically phenols constituted more than 90% of the TC in WSFs and that other relatively insoluble chemical classes probably contributed little to acute toxicity. For the studies reported here, phenol composition of WSFs varied somewhat according to process type and/or boiling range. However, in most cases, phenols, cresols and C_2 phenols predominated. Thus differences in relative solubilities (e.g. amount of TC in WSF) among parent materials may be attributable to differences in concentrations of lower-molecular-weight constituents. The low solubility of constituents of reference fuel oils (i.e., petroleum) is reflected in WSFs that have low TC concentrations.

Results of toxicity studies with WSFs from coal liquids, shale oil, and crude and refined petroleum are summarized in Figure 1. Based on the percent WSF needed to elicit the 48 h LC_{50} (reflecting both solubility and inherent toxicity), the ITSL and EDS coal liquids were less hazardous to *D. magna* than SRC-II liquids but more hazardous than petroleum materials. Based on mg/L TC in solution, the ITSL material exhibited lower toxicity than all but the No. 2 fuel oil and Prudhoe Bay crude. The No. 6 fuel oil WSF was the most toxic material tested, based on mg/L TC in solution required to elicit a toxic response, but because of low solubility was less hazardous than the synfuel materials.

Toxicity of fossil-derived materials to other aquatic organisms can also be compared. Giddings and Washington (1981) evaluated a wide range of petroleum and coal liquefaction materials and found that, based on percent dilution of the WSF, the potential hazard of petroleum products to algae was less than that of coal liquids. Gray et al. (1982) and States et al. (1981) evaluated SRC-II WSFs and reported similar results after chronic exposure of algae and daphnids, respectively. Inherent toxicity was mainly related to the presence of highly soluble phenolic constituents in the coal liquid WFS. Differences in toxicities among materials with similar solubilities (i.e., TC in WSF) may reflect compositional differences in WSFs.

Based on the percent WSF needed to elicit a toxic response, the lower-boiling range EDS and SRC II liquids were the most hazardous (Table 1). However, based on mg/L TC in solution, the higher-boiling-range coal liquids may be inherently more toxic than their lower boiling-range counter-parts. Other studies showed that higher-boiling-range coal liquids pose the greatest potential health problems (mutagenicity, carcinogenicity), while lower-boiling-range materials are of lesser concern (Gray and Drucker 1981, Gray and Cowser 1982, Gray 1984). Also of note is the finding that the acute hazard to Daphnia of the SRC-II WSF from material with a boiling range of 300° to 850°F reflects the hazard of material boiling from 300° to 700°F. That is, the acute hazard of the lower-boiling-range, 300° to 700°F cut, was not decreased by inclusion of the less hazardous, less soluble higher-boiling-range, 700° to 850°F cut.

Data for an EDS process solvent derived from three different coal types show similar trends with respect to solubility, inherent toxicity, and acute hazard to Daphnia (Table 2). Based on percent WSF needed to elicit a toxic response, coal liquid derived from Texas lignite was least hazardous. However, based on mg/L TC in solution, there were no differences in inherent toxicity that could be related to coal type. It is noteworthy that operation of a coal liquefaction facility is generally optimized according to the particular coal being used and, thus, it is not possible to directly evaluate the influence of coal type on toxicity. Instead, studies of this type may only reveal similarities or differences among materials derived from processes that have been optimized for the coal being processed.

Because all materials evaluated in these studies were treated identically, each had equal opportunity to enter solution. Toxicity of WSFs of each material was, therefore, highly dependent on the overall solubility of the parent material and the toxic properties of individual soluble components (Gray et al. 1983). Other factors known to influence toxicity of complex organic materials to daphnids include mixing regimes (Bean et al. 1981) and exposure conditions (Becker and Crass 1982), including water temperature (Ullrich and Millemann 1983) and water quality (Becker et al. 1983).

Since all WSFs tested were complex mixtures of several organic compound classes, toxicity cannot be attributable to any one chemical class. Instead, biological responses resulted from exposure to interacting toxic components. WSFs derived from coal liquids contained lower-molecular-weight phenolics as a major contributor to the TC in solution and were more toxic to daphnids than were phenol or various cresols tested as separate compounds (De Graeve et al. 1980).

In summary, concentrations of water-soluble components varied with composition and solubility of parent material under similar mixing conditions. The acute hazard to Daphnia magna reflected solubility of the parent material and, in most cases, could be predicted from TC concentrations in solution. Given similar toxicities to aquatic organisms, release (e.g. a spill) of small amounts of highly soluble organic materials may pose a greater immediate hazard than the release of larger volumes of relatively insoluble materials. However, release of less soluble, but more persistent compounds such as higher-molecular-weight phenols and aromatic hydrocarbons will remain as a long-term environmental problem (Gray et al. 1982, 1983). Because of their higher solubility, full-boiling-range coal liquids pose a greater potential acute hazard in aquatic habitats than shale oil or petroleum materials. Although higher-boiling-range coal liquids pose the greatest potential human health hazard (Gray and Drucker 1981; Gray and Cowser, 1982; Gray 1984), lower-boiling-range coal liquids were most soluble in water, and some components (e.g. phenolics) are acutely toxic. Potential differences in acute toxicity among coal liquids derived from different coals were masked by optimized process conditions. The coal type, processed under conditions optimized for its specific characteristics, appears to have little influence on inherent toxicity of coal-derived liquids, although the latter may differ in their solubility in water, and therefore, their acute hazard.

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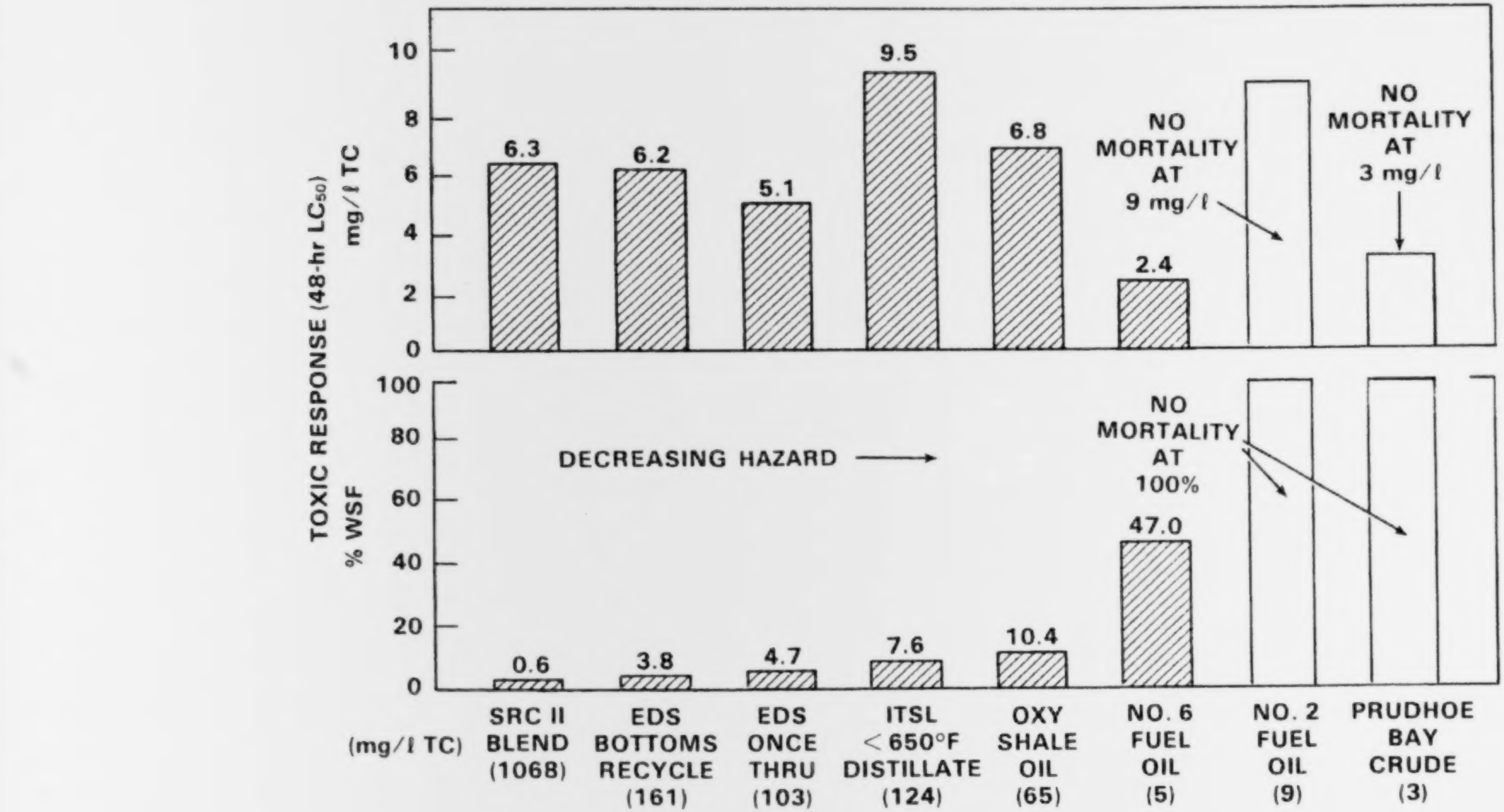

Figure 1. Inherent toxicity and acute hazard to Daphnia magna of coal liquids, shale oil, and reference fuel oils expressed as mg/L total carbon (TC) and % water-soluble fraction (WSF) eliciting a toxic response (LD_{50}), respectively. Numbers in parentheses indicate relative concentrations (mg/L TC) of the respective WSFs (derived from Dauble et al. 1983b).

Table 1. Solubility, inherent toxicity and acute hazard to *Daphnia magna* of three different boiling range and SRC-II EDS coal liquid WSFs. Values are expressed as means of duplicate tests.

Process	Boiling Range (°F)	WSF Concentration (mg/L)	48 h LC_{50}	
			mg/L TC	%WSF
SRC-II	300-700	417	3.1	0.8
	300-850	300	2.4	0.8
	700-850	2	1.0	51.3
EDS	150-400	730	5.6	0.8
	400-700	220	6.9	3.1
	700-800	5	2.1	47.1

Table 2. Solubility, inherent toxicity and acute hazard to *Daphnia magna* of EDS process solvent (bottoms recycle operations) derived from three different coals. Values are expressed as mean ± S.D.

Coal Type	WSF concentration (mg/L TC)	48 h LC_{50}		N
		mg/L TC	%WSF	
Illinois No. 6	123 ± 41	6.4 ± 1.8	5.8 ± 2.5	5
Wyodak	89 ± 12	6.7 ± 0.7	7.7 ± 1.7	3
Texas Lignite	58 ± 11	6.7 ± 1.1	13.1 ± 2.6	3

A REVIEW OF THE IMPACTS AND RECOVERY OF INTERTIDAL HABITATS AND COMMUNITIES FOLLOWING ACCIDENTAL OIL SPILLS

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A recent review of worldwide oilspill case histories and followup studies has indicated that intertidal habitats and organisms are frequently the resources which have been most visibly affected following oil spills. The impacts and recovery of intertidal communities following these events has varied widely depending on the circumstances surrounding the spill and the characteristics of the intertidal habitat and community affected. This paper examines the contribution of some of these factors to the impact and recovery of intertidal habitats and communities. Some of the most significant factors affecting oil spill impacts and subsequent recovery of intertidal communities are: [1] the time of year of the event, [2] the type and volume of oil reaching the shoreline, [3] its physical/chemical nature following weathering both at sea and on the shoreline, [4] the degree and vertical extent of intertidal contamination, [5] the composition of the substrate, [6] exposure to wave action, and [7] the shoreline restoration methods used in the cleanup response. In general, the organisms on rocky shorelines in areas exposed to strong wave action have recovered most rapidly, while recovery has required ten years or more in areas where oil has penetrated and persisted in fine granular substrates. Impacts and recovery of sedentary organisms have often been more severe and long-term than those reported for mobile species.

UNE REVUE DES IMPACTS DE DÉVERSEMENTS DE PÉTROLE ACCIDENTELS SUR LES HABITATS ET COMMUNAUTÉS INTERTIDAUX ET LEURS RÉCUPÉRATIONS SUBSÉQUENTES

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Une revue récente des cas historiques de déversements de pétrole dans le monde entier et des études qui suivirent indiquent que les habitats et les organismes intertidaux sont souvent les ressources le plus visiblement affectées à la suite de déversements de pétrole. Les impacts et la récupération des communautés intertidales à la suite de ces événements varient largement selon les circonstances entourant les déversements et selon les caractéristiques de la communauté et de l'habitat intertidal affecté. Cet article examine la contribution de quelques uns de ces facteurs à l'impact et à la récupération des habitats et communautés intertidaux. Quelques uns des facteurs les plus importants quant à l'impact des déversements de pétrole et la récupération subséquente des communautés intertidales sont: [1] le temps de l'année de l'événement, [2] le type et le volume de pétrole approchant le rivage, [3] les propriétés physiques/chimiques de l'événement suivant le désagrègement à la mer et sur le rivage, [4] le degré et l'étendue verticale de la contamination intertidale, [5] la composition du substrat, [6] le degré d'exposition à l'action des vagues, et [7] les méthodes de restoration de rivage utilisées durant le nettoyage. En général, les organismes des rivages rocheux dans les régions exposées à des fortes actions de la part des vagues récupèrent le plus rapidement, tandis que la récupération demande jusqu'à dix ans et plus dans les régions où le pétrole a pénétré et persiste dans les substrats granuleux fins. La récupération et les impacts sur les organismes sédentaires sont souvent plus sévères et à plus long terme que ceux rapportés pour les espèces mobiles.

FISH, FISHERIES AND OIL POLLUTION: MYTHS AND REALITIES

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On a worldwide basis, the amounts of petroleum hydrocarbons entering the marine environment from oil spills is relatively low compared with total inputs from a variety of sources, including natural oil seeps and runoff from large coastal cities. When marine disasters involving the spillage of large amounts of oil have occurred, there has been little or no long-term ecological impact except in localized animal populations near shore. There is also no evidence to suggest that fish stocks have been affected by oil pollution. Fish larvae are especially sensitive to oil pollution but it is indicated that very large proportions of larvae would have to be destroyed before effects are translated into population effects or fish "catch" losses. The worst situation would seem to be an uncontrolled blowout during spawning when large quantities of larvae may be found in surface waters. Many components in petroleum appear to be well tolerated by marine organisms but biochemical and physiological responses have been observed, and these indices will be useful for monitoring biological effects around offshore petroleum development sites. In reference to the use of drilling muds, sublethal effects on benthic organisms will likely be confined to very small areas - probably no more than tens or hundreds of meters from the rig sites. In the case of ideal current conditions (for worse case scenarios), it would seem difficult to invoke a significant level of hazard beyond a few kilometers. All in all, it is concluded that many of the draconian perceptions of the probable environmental impacts of oil pollution have a most unlikely reality and it appears that problems associated with gear fouling and fish tainting will be of more importance, for both fish and fisheries interests.

POISSONS, PÊCHERIES ET POLLUTION PÉTROLIFÈRE: MYTHES ET RÉALITÉ

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Sur une base mondiale, les quantités d'hydrocarbures de pétrole entrant dans l'environnement marin à travers des déversements de pétrole sont relativement basses comparées au total provenant d'autres sources, parmi lesquelles on retrouve les échappements de pétrole naturel et les écoulements de grandes villes côtières. Quand des désastres marins impliquant le déversement de larges quantités de pétrole surviennent, il n'y a que peu ou pas d'impact écologique à long terme, excepté sur les populations d'animaux situées près de la rive. Il n'y a aussi aucune évidence qui permet de suggérer que les réserves de poissons soient affectées par la pollution provenant du pétrole. Les larves de poisson sont extrêmement sensibles à la pollution pétrolifère mais il semble qu'une très grande proportion des larves doit être détruite avant que les effets ne se traduisent en effets sur la population ou en perte de récolte de poissons. La pire situation semble être un échappement de pétrole hors de contrôle durant la fraye, quand de grandes quantités de larves peuvent être trouvées dans les eaux de surface. Plusieurs composés à base de pétrole paraissent être bien tolérés par les organismes marins, mais des réponses biochimiques et physiologiques ont été observées, et les indices en résultant seront utiles pour suivre les effets biologiques autour des sites de développements pétrolifères en haute mer. En ce qui a trait aux boues de forage, les effets sub-lêtaux sur les organismes benthiques seront probablement limités à de petites régions - probablement pas plus que dix ou cent mètres autour de la plateforme de forage. Dans le cas de conditions de courant idéales (en mettant les choses au pire), il semble difficile d'en arriver à un niveau significatif de danger au-delà de quelques kilomètres. Pour terminer, on peut conclure que plusieurs des perceptions draconiennes à propos des impacts environnementaux possibles de pollution par pétrole sont irréalistes, et il semble probable que les problèmes d'équipement encrassé et de poissons infectés prendront de plus en plus d'importance, et ceci dans l'intérêt et des poissons et des pêcheries.

CONTRIBUTED PAPERS

A LABORATORY APPARATUS FOR STUDYING ORGANISMS IN STRATIFIED WATERS

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An acrylic tank is described, in which a 2.25 m high, 0.75 m wide, 2.0 m long body of water, may be stratified into three horizontal 75 cm layers. Within each layer, temperature, salinity, dissolved oxygen and water velocity may be varied. A metal-halide light source with dawn-dusk control provides light to the apparatus via a 'light pipe'.

The apparatus is primarily used to study fish behaviour in a simulated, vertically stratified estuarine water column: fresh water in the surface layer, overlying two layers of greater salinity. In each layer salinity control is obtained through the proportional mixing of fresh (well) water and sea water; temperature is controlled by the use of heat exchangers (heat input is continuously provided from pumps which circulate the water) and water velocities (to 6 cm/s) are regulated by operation of butterfly valves associated with orifice meters and differential pressure indicators in the pipes delivering water to the tank. Dissolved gasses are varied by the use of gas stripping/aeration columns through which water passes to reservoirs before admission to the apparatus.

The effect of pollutants upon fish behaviour will be studied in relation to the above-mentioned variables.

UN APPAREIL DE LABORATOIRE POUR ÉTUDIER DES ORGANISMES DANS DES EAUX STRATIFIÉES

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On décrit un réservoir en acrylique dans lequel une nappe d'eau de 2.25 m de haut, 0.75 m de large et de 2.0 m de long peut être stratifiée en trois couches horizontales de 75 cm. La température, salinité, l'oxygène dissous et la vélocité de l'eau peuvent être variés dans chaque couche. Une source de luminère à halogène-métal avec un contrôle noirceur-lumière fournit la lumière à l'appareil via un "tuyau à lumière".

L'appareil est surtout utilisé pour étudier le comportement de poissons dans une simulation de colonne d'eau d'estuaire stratifiée verticalement: de l'eau douce dans la couche de surface recouvre deux couches d'eau à plus grande salinité. Dans chaque couche, le contrôle de la salinité est obtenu à travers le mélange proportionnel d'eau douce (d'un puits) et d'eau de mer; la température est contrôlée par l'utilisation d'échangeurs de chaleur (l'énergie calorifique est continuellement fournie par des pompes qui font circuler l'eau) et la vélocité de l'eau (jusqu'à 6 cm/s) est contrôlée par l'opération de valves papillon associées avec des compteurs situés à l'orifice et par des indicateurs de pression différentielle dans les tuyaux fournissant l'eau au réservoir. Les gaz dissous sont variés par l'utilisation de colonnes d'enlèvement/aération de gaz à travers lesquelles l'eau passe vers les réservoirs avant d'être admise dans l'appareil.

Les effets des substances polluantes sur le comportement des poissons seront étudiés en relation avec les variables ci-haut mentionnées.

This apparatus was designed to examine fish behaviour under simulated estuarine conditions. The natural surface-water orientation of juvenile salmon should facilitate the study of behavioural responses to pollutants and variations in dissolved oxygen concentrations within a vertically stratified water column.

The apparatus was constructed by Western Canada Hydraulics Ltd., and Department of Fisheries and Oceans staff at the West Vancouver Laboratory. Testing of the apparatus is in the final stages and accordingly we cannot report experimental results at this time. However, the following comments provide a brief description of the apparatus and some of the significant features are shown in Figure 1.

An acrylic tank (2.4 x 2.4 x 0.8 m; 12.7 mm wall thickness) is used to produce a stratified water column. Stratification is typically achieved by the admission of fresh (well) water over one or two layers of more saline water. The volume of each of the three layers is approximately 1500 L.

Well water and salt water are admitted to the apparatus after passing through two insulated ABS aeration/de-aeration columns (10 cm x 2.5 m) packed with 2.5 cm "Biorings", which discharge into 130-L constant head reservoirs. For each of the three zones in the apparatus, flow meters (0 to 38 L/min) permit the proportional mixing or separation of fresh and salt water. Ninety percent replacement of the water in each zone can be achieved in less than 2 h at a flow rate of about 30 L/min.

Water pumps, with a capacity of about 2000 L/min drive water through each of the three zones in the experimental tank. Heat added by pumps and friction within the system is balanced by passing a portion of the recirculating water through heat exchangers cooled by a flow of municipal water. Filters (particulate and charcoal) are located in this part of the apparatus (the "bypass loop").

Main control valves regulate the flow of water into the three zones of the tank. Pressure differences along these 15 cm diameter service pipes are recorded on gauges and the readings are proportional to the velocity of water movement in the tank. During calibration tests by the use of dye injections, laminar water flows up to 12 cm/s were attained. However, water velocities between 3 and 4 cm/s are usually used to minimize mixing between adjacent water layers. Two standpipes are located along each of the water pipes servicing the tank. One standpipe is for the admission of material, or monitoring equipment, while the other is for water level control. Adjustment of the water level control standpipes by a worm-gear changes the level of the interface between adjacent water bodies and also serves to continuously drain the apparatus.

Temperature, conductivity and dissolved oxygen are monitored in each recirculating water loop just before discharge into the tank. Upon entry, the water is distributed over a fixed depth through acrylic screens which serve to even the flow. Layering of fresh water over salt water can be readily achieved, and is facilitated by the use of acrylic planes set at the interface of adjacent water layers. Water passes across each zone and exits via centrally positioned 15 cm pipes and thereafter is recirculated in the system.

Table 1 provides information that demonstrates the stability of the stratified water column between 0800 and 1600 h, Nov. 7, 1984. We have been able to maintain such stratified conditions over a number of days.

Table 1. Changes in salinity, temperature and dissolved oxygen between 0800 h and 1600 h, November 7, 1984.

	Water layers					
	Top		Middle		Bottom	
	n	± S.D.	n	± S.D.	n	± S.D.
Temperature (°C)	13	12.4±0.1	13	12.4±0.1	13	12.5±0.1
Salinity (ppt)	14	0.6±0.1	14	22.0±0.7	14	23.8±0.3
Dissolved oxygen (mg/L)	14	10.3±0	14	9.5±0	14	9.1±0.1
Water velocity (cm/s)		3-4		3-4		3-4
Replacement water flow (L/min)						
Fresh (<0.1 ppt)		31.5		-		-
Salt (22.5-27.2 ppt)		-		18.0		13.5

Air bleeds are located in many parts of the apparatus to ensure that air escapes prior to and during experimentation. We have been able to avoid gas supersaturation in the apparatus with appropriate temperature regulation and use of the air bleeds.

A 400 watt metal-halide light source transmits light to the tank from above. A "Light Pipe^{TM}" is used for this purpose and screening of the light by the use of a motor driven screen provides photoperiod as well as seasonal dawn/dusk control. Light intensities are about 1/20th of bright sunlight and are about five times greater than those measured on a dull and rainy day.

The experimental tank area can be surrounded by drapes and monitoring of fish behaviour will be carried out by a time lapse video recorder coupled to a high sensitivity/resolution T.V. camera (0.1 lux, 800 lines) and monitor.

ACKNOWLEDGMENTS

G.E. Piercey, C. McPherson and J. Bunn assisted in the presentation of the poster. H. Herunter and R. Lauzier deserve special mention for their efforts to modify and test the apparatus. D. Price typed this abstract.

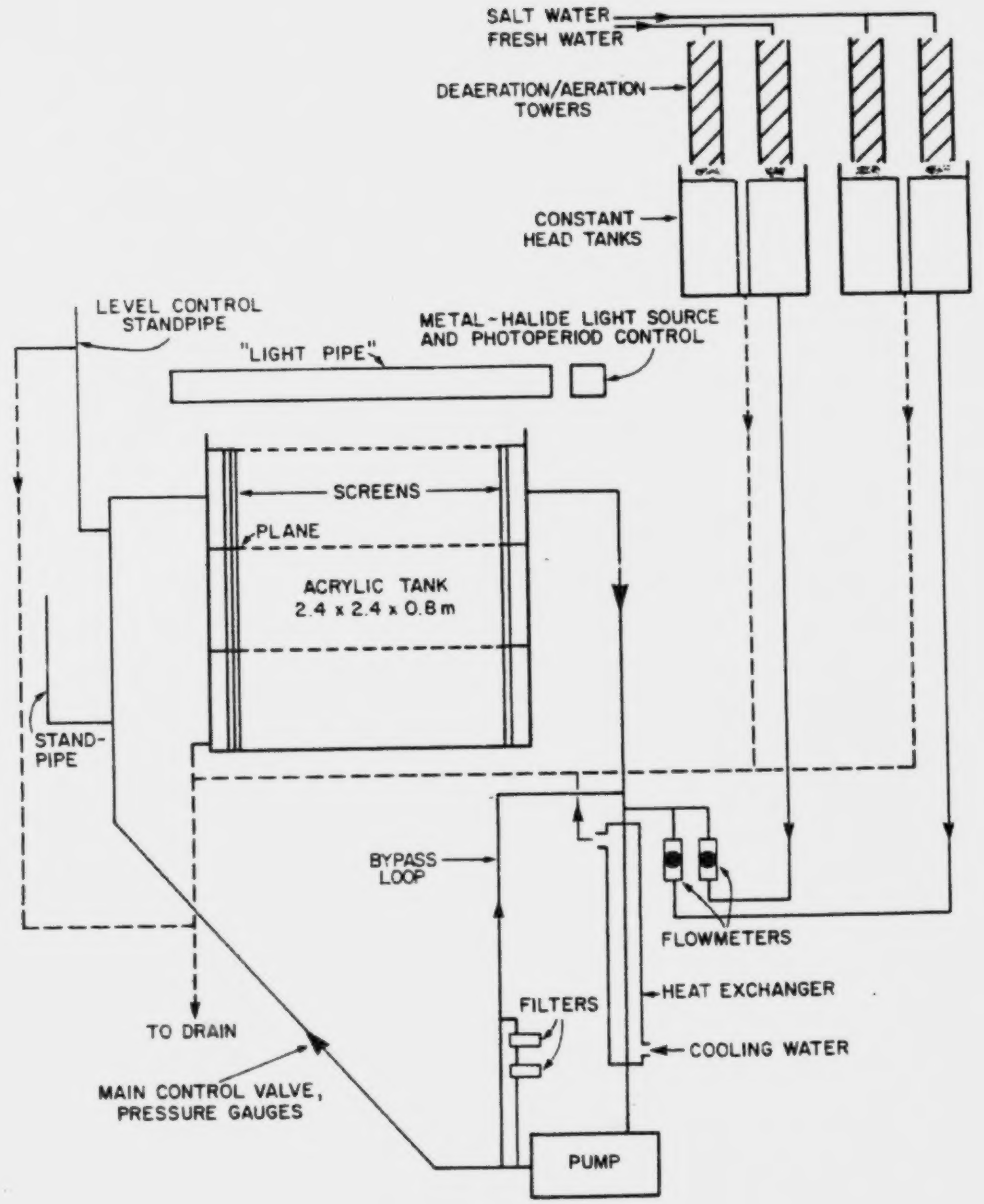
SALT WATER
FRESH WATER
DEAERATION/AERATION TOWERS
CONSTANT HEAD TANKS
LEVEL CONTROL STANDPIPE
METAL-HALIDE LIGHT SOURCE AND PHOTOPERIOD CONTROL
"LIGHT PIPE"
SCREENS
PLANE
ACRYLIC TANK 2.4 x 2.4 x 0.8 m
STAND-PIPE
BYPASS LOOP
FLOWMETERS
HEAT EXCHANGER
FILTERS
COOLING WATER
TO DRAIN
MAIN CONTROL VALVE, PRESSURE GAUGES
PUMP

Figure 1. Diagram of the main components comprising the water column simulator (one of three loops in the system is shown).

SEDIMENT BIOASSAY TESTS PROVIDE TOXICITY DATA NECESSARY FOR ASSESSMENT AND REGULATION

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Bioassays involving laboratory exposure of test species to field-collected sediments are assuming importance in toxicological assessments conducted in the United States. Because sediments are a major repository for persistent aquatic contaminants, sediment bioassays provide information on the amount of biologically active substances by the level of their effect on test organisms. Such information cannot be derived by water column toxicity tests or by chemical analyses of the sediments. Although analyses of resident benthic communities may provide this information, the results are often inconclusive due to such factors as their large-scale natural variability.

A variety of sediment bioassay techniques have been developed and used to date, primarily in the marine environment of Puget Sound. Of these, four specific tests appear to be the most useful: 10-d survival tests using the amphipod *Rhepoxynius abronius* provide information on acute lethality; 48-h oyster larvae development tests and oligochaete respiration response tests provide information on sub-lethal effects; fish cell anaphase aberrations (determined *in vitro*) provide information on genotoxicity. Present Canadian regulations controlling the identification and disposal of contaminated sediments are based on chemical analyses and as such provide no information on bioavailability or the potential of contaminated sediments to affect aquatic biota. Sediment bioassays provide this information and, as such, should be included in assessment and regulation activities in Canada. An overview of sediment bioassay tests and results to date is presented in support of this contention.

DES TESTS DE BIOASSAI SUR DES SÉDIMENTS FOURNISSENT DES DONNÉES DE TOXICITÉ NÉCESSAIRES À L'ESTIMATION ET LA RÉGULATION TOXICOLOGIQUE

Chapman, Peter M. E.V.S. Consultants, 195 Pemberton Avenue, North Vancouver, B.C. V7P 2R4

Plusieurs techniques de bioassais sur sédiments impliquant l'exposition en laboratoire de certaines espèces à des sédiments collectés sur le terrain ont été développées dans des programmes d'estimation toxicologique menés aux États-Unis. Etant donné que les sédiments constituent des dépôts majeurs pour les contaminants aquatiques qui persistent dans l'environnement, les bioassais de sédiments fournissent des informations sur la quantité de substances biologiquement actives par l'ampleur de leurs effets sur les organismes étudiés. Quatre tests se sont avérés des plus utiles dans l'environnement marin: les tests de survie de 10 j utilisant l'amphipode *Rhepoxynius abronius* fournissent des informations sur la mortalité extrême, les tests de développement de 48 h de larves d'huîtres et les tests sur la réponse respiratoire d'oligochètes fournissent des informations sur les effets sous-létaux, et les aberrations de cellules de poissons en anaphase (déterminées *in vitro*) fournissent des informations sur la génotoxicité. Les tests sur la survie et sur le cycle de vie de *Daphnia* paraissent être les techniques de bioassai les plus importantes développées jusqu'à date dans le milieu d'eau douce. Les présents règlements canadiens contrôlant l'identification et la disposition de sédiments contaminés sont basés sur des analyses chimiques et comme tels ne fournissent pas d'information sur la disponibilité de l'environnement ou sur le potentiel contaminant des sédiments en milieu aquatique. Les bioassais de sédiments fournissent cette information et devraient donc être inclus dans les activités d'estimation et de régulation au Canada. Un aperçu général des tests et des résultats obtenus jusqu'à date est présenté en support de cette assertion.

INTRODUCTION

Discharge of chemicals and waste materials into the aquatic environment results in sediment accumulations of these materials. Although such accumulations may include several hundred organic and inorganic compounds (Malins et al., 1984), present Canadian regulations governing the identification and disposal of contaminated sediments are based solely on chemical analyses for a few selected chemicals. The present reliance on chemical measurements does not provide necessary data for environmental assessment because: [1] available technology does not allow quantification of all possible chemicals in the sediments, nor are more than a narrow range of chemicals analyzed; and, [2] determination of the presence and concentrations of chemicals in sediments provides no information on bioavailability or toxicity. As a result, under present regulations, non-toxic sediments may be incorrectly evaluated as being of concern, while toxic sediments may be assessed as harmless.

Benthic community structure analysis, although expensive, can be used to determine toxic sediment-related effects. However, these data must be interpreted in the light of the fact that benthic communities have a high natural variability resulting from factors other than contaminant effects (Beanlands and Duinker, 1983). As a result, additional chemical and toxicological data are required to relate benthic community effects to chemically contaminated sediments. The one tool that readily provides the needed toxicity data for assessment and regulation and which, in the context of complementary chemical and ecological data sets, also provides direct proof of contaminant effects (positive or negative), is the sediment bioassay.

Sediment bioassays are distinctly different than water column bioassays which usually involve effluent and receiving water tests, and which have not shown good relationships to benthic community structure nor to sediment chemistry measures. Sediment bioassays relate well to these environmental parameters, and should be applied for receiving environment studies, monitoring of dredging and clean-up, and ocean dumping assessment.

Sediment bioassays are conducted by exposing biota or biological systems in the laboratory to field-collected sediments. These bioassays meet Brinkhurst's (1983) criterion of: "simple toxicological procedures (that) provide a useful forensic tool". The purpose of the present paper is to demonstrate that sediment bioassays provide essential data for the assessment and regulation of contaminated sediments. To this end, the paper reviews methodologies and interrelationships with sediment chemistry and *in situ* biological effects, discusses the relevance and uses of sediment bioassays and recommends the use of specific sediment bioassay techniques.

SEDIMENT BIOASSAY METHODS

Marine environment

Sediment bioassays have been used in various areas of the United States to determine the relative toxicity of sediment samples (e.g. Baltimore Harbor - Tsai et al., 1979; New York Bight - Wurster, 1982; Teitjen and Lee, 1984). The most extensive testing to date has been undertaken in Puget Sound, Washington state, as summarized in Table 1 and discussed below. Additional details of sediment bioassay testing in Puget Sound and in other areas of the United States are provided by Swartz (1984).

Although high levels of chemical contaminants have been found in particular areas of Puget Sound (Malins et al., 1980, 1982; Long, 1983) only the most sensitive species are killed rapidly and directly by exposure to contaminated sediments in the laboratory. Sensitive species include the grass shrimp, Palaemonetes pugio (Shuba et al., 1978), the clam Macoma inquinata (Swartz et al., 1979), and the phoxocephalid amphipods Grandifoxus grandis (Pierson et al., 1983) and Rhepoxynius abronius (Swartz et al., 1979, 1981, 1982, in press a; Ott et al., in prep.; Chapman et al., 1982a, b, 1984; Chapman and Fink, 1983).

The most sensitive species used in acute lethal sediment bioassay tests in Puget Sound is the phoxocephalid amphipod Rhepoxynius abronius (Chapman et al., 1982a; Chapman and Fink, 1983; R. Swartz and J. Cummins, EPA, pers. comm.). This sensitivity is consistent with field observations that this species and phoxocephalids as a group are not found in contaminated areas (Swartz et al., 1982; Comiskey et al., 1983).

Sublethal bioassays including partial or complete life-cycle tests have also been conducted in Puget Sound. Extensive sublethal testing has been done using the respiratory response of oligochaetes (Monopylephorus cuticulatus) exposed to sediment elutriates (Chapman et al., 1982a, b, 1984; Chapman and Fink, 1983). Testing for reproductive impairment effects by Chapman et al. (1983, in press a) has involved partial life-cycle tests with oyster larvae, (Crassostrea gigas) and surf smelt (Hypomesus pretiosus pretiosus) and full-cycle tests with the polychaete Capitella capitata. Of these three test methods, the surf smelt showed such high natural variability that it was difficult to distinguish toxicity-related effects. Both the C. capitata and oyster larvae tests were effective in determining sediment toxicity, however the oyster larvae were more sensitive than the polychaetes, the latter exhibiting a rapid adaptive response to contaminated whole sediments and elutriates (Chapman et al., 1983). Laboratory tests designed to demonstrate histopathological effects to English sole were not effective (McCain et al., 1982).

The most sensitive sublethal sediment bioassay is the oyster larvae partial life-cycle test (Chapman et al., 1983, 1984; Chapman and Morgan, 1983). The oligochaete respiration response test is slightly less sensitive (Chapman et al., 1984).

Bioassays for cytotoxic and genotoxic responses in cultured fish cells have been conducted using extracts of Puget Sound sediments. These have proven to be extremely sensitive indicators of sediment toxicity, although they are so sensitive that "natural" as well as anthropogenic toxic responses are detected (Chapman et al., 1982a, 1984; Landolt and Koran, 1984).

Freshwater environment

Sediment bioassay testing has been less extensive in fresh than in marine waters. A full review of freshwater sediment bioassays is provided by Nebeker et al. (1984), and recent testing is summarized in Table 2.

Table 1. Summary of laboratory bioassay tests with Puget Sound sediments.

Taxa	Investigator(s)	Comments
ACUTE LETHAL BIOASSAYS		
Annelid Worms:		
Monopylephorus cuticulatus	Chapman et al. (1982a)	not sensitive
Glycinde picta	Swartz et al. (1979)	not sensitive
Crustaceans (Copepods):		
Acartia tonsa	Shuba et al. (1978)	not sensitive
Tigriopus sp.	Shuba et al. (1978)	not sensitive
Crustaceans (Amphipods):		
Eogammarus confervicolus	Chapman et al. (1982a)	relatively insensitive
Eohaustorius washingtonianus	Ott et al. (in prep.)	relatively insensitive
Grandifoxus grandis	Pierson et al. (1983)	sensitive
Rhepoxynius abronius	Swartz et al. (1979, 1981 1982, in press a); Ott et al. (in prep.); Chapman et al. (1982a, b, 1984); Chapman and Fink (1983)	very sensitive
Crustaceans (Cumaceans):		
several species	Swartz et al. (1979)	not sensitive
Crustaceans (Shrimp):		
Palaemonetes pugio	Shuba et al. (1978)	sensitive
Bivalve Molluscs:		
Macoma inquinata	Swartz et al. (1979)	sensitive
Protothaca staminea	Swartz et al. (1979)	not sensitive
Rangia cuneata	Shuba et al. (1979)	not sensitive

Table 1 cont...

Taxa	Investigator(s)	Comments
Fish:		
Gasterosteus aculeatus (three-spine stickleback)	Chapman et al. (1982a)	not sensitive
Oncorhynchus kisutch (coho salmon)	Legore and DesVoigne (1973)	not sensitive
Oncorhychus tsawytscha (chinook salmon)	Pierson et al. (1983)	not sensitive
SUBLETHAL BIOASSAYS		
Respiration rate of the oligochaete worm, *Monopylephorus cuticulatus*	Chapman (1984); Chapman et al. (1982a, b, 1984, in press a); Chapman and Fink (1983)	very sensitive
Oyster larvae, *Crassostrea gigas*, partial life-cycle bioassay	Chapman et al. (1983, in press a); Chapman and Morgan (1983); Pierson et al. (1983)	very sensitive
Surf smelt, *Hypomesus pretiosus pretiosus*, partial life-cycle bioassay	Chapman et al. (1983)	excessive natural variability
Full life-cycle bioassay with the polychaete worm, *Capitella capitata*	Chapman et al. (1983) Chapman and Fink (1984)	sensitive
Histopathological measures of toxicity to English sole, *Parophrys vetulus*, with 3 mo. sediment exposure	McCain et al. (1982)	not sensitive
CYTOTOXIC/GENOTOXIC BIOASSAYS		
Cell proliferation *in vitro*	Chapman et al. (1983, 1984, in press a)	very sensitive
Mitotic abnormalities (anaphase aberrations) *in vitro*	Chapman et al. (1982a, b, 1984)	very sensitive

Table 2. Summary of recent laboratory bioassay tests with freshwater sediments.

Taxa	Investigator(s)	Comments
ACUTE LETHAL BIOASSAYS		
Crustaceans (Cladoceran):		
Daphnia magna	Prater and Anderson (1977); Leonard (1983); Malueg et al. (1984a, b); Nebeker et al.; (1984); Schuytema et al. (1984)	very sensitive
Crustaceans (Amphipods):		
Hyalella azteca	Nebeker et al. (1984)	not sensitive
Gammarus lacustris	Nebeker et al. (1984)	not sensitive
Insects (Midge):		
Chironomus tentans	Nebeker et al. (1984)	not sensitive
Insects (Mayfly):		
Hexagenia limbata	Prater and Anderson (1977); Nebeker et al. (1984); Malueg et al. (1984a, b)	not sensitive
SUBLETHAL BIOASSAY		
Chironomus tentans larval survival, growth and adult emergence	Nebeker et al. (1984)	under evaluation
Daphnia magna life-cycle bioassay	Nebeker et al. (1984)	very sensitive
Hyalella azteca partial life-cycle bioassay	Nebeker et al. (1984)	recovery of young is difficult
CYTOTOXIC/GENOTOXIC BIOASSAYS		
Mitotic abnormalities (anaphase aberrations) *in vitro*	E.V.S. Consultants, unpub. data	very sensitive

As is the case with marine studies, most species tested did not respond in acute lethal tests. However, Daphnia magna has been used successfully as a sediment bioassay "white rat" in a number of studies (Prater and Anderson, 1977; Leonard, 1983; Malueg et al., 1984a, b; Schuytema et al., 1984).

Sublethal bioassays are in the process of development. Currently, the most sensitive sublethal bioassay involves life-cycle testing with Daphnia magna (Nebeker et al., 1984). Bioassays for cytotoxic and genotoxic responses in cultured fish cells have been conducted in a similar manner to marine sediments, and have proven similarly sensitive in fresh as in marine sediments (E.V.S. Consultants, unpub. data).

INTERRELATIONSHIPS BETWEEN SEDIMENT CHEMISTRY AND TOXICITY

Marine environment

Correlation of sediment bioassay results with total contaminant levels have met with more success than correlations with individual sediment contaminant concentrations. Tsai et al. (1979) determined 48 h LC_{50} values for the mummichog (Fundulus heteroclitus), spot (Liostomus xanthus) and a mollusc (Mya arenaria) exposed to constantly mixed Baltimore Harbor sediment concentrations of between 79 and 0.63 g/L. There was no correlation between bioassay results and measured concentrations of Pb, Cr, Zn, Cd, Hg, Ni, Cu, Mn, As and PCBs in the sediment. Tsai et al. (1979) concluded that no single chemical contaminant accounted for sediment toxicity.

Similar results have been obtained for the New York Bight. Wurster (1982) tested eight sediment samples from the Hudson-Raritan estuary using a two species phytoplankton bioassay. He found no correlation between bioassay results and PCB and PAH concentrations, but did note some correspondence with levels of total heavy metals in sediments.

Tietjen and Lee (1984) used free-living nematodes as bioassay organisms to determine the relative toxicity of eight sediment samples from the New York Bight representing a gradient of stations from lightly to heavily impacted by pollution. Sediment contaminants measured were PCBs, PAHs, Cd, Cr, Cu, Hg, Pb and Zn. The natural daily increase in number of nematode generations was used as a measure of sediment quality. Population growth was found to be less than half when PCB concentrations were greater than 270 ppb, and when PAH concentrations were greater than 8700 ppb.

In Puget Sound, various investigators (i.e. Comiskey et al., 1983; Quinlan et al., in press; Chapman et al., in press b) have attempted unsuccessfully to correlate sediment bioassay results with particular individual sediment chemical contaminants. However, a good correspondence has been noted between overall levels of sediment chemical contamination (total metals, and total PAHs and PCBs) and sediment bioassay results (Quinlan et al., in press; Chapman et al., in press b).

In British Columbia, Chapman and Barlow (1984) found that sediment toxicity did not correspond with high levels of individual metals in sediments. Of particular interest, these authors noted that in some cases toxic effects were recorded when sediment levels of Cd and Hg, which are regulated under the Ocean

Dumping Control Act (ODCA), were below the regulatory criteria; in other cases, toxicity was not recorded when the chemical criteria were exceeded.

Freshwater environment

Sediment bioassays conducted to date in the freshwater environment have been principally concerned with areas of heavy metal contamination. In these areas, excellent correspondence has been noted between sediment bioassay results and sediment levels of particular metals. For instance, Malueg et al. (1984a) conducted sediment bioassays in two areas of the Keweenew Waterway, Michigan, and reported positive correlations between mortality in bioassays and the Cu content of the sediment. Similar conclusions were noted by Malueg et al. (1984b) in studies conducted in three other metal-contaminated areas of the United States (Wisconsin, Michigan and California).

INTERRELATIONSHIPS BETWEEN IN SITU BIOLOGICAL EFFECTS AND SEDIMENT TOXICITY

Marine environment

Comparisons between the results of sediment bioassays and in situ biological effects (e.g. benthic infaunal changes, histopathological abnormalities in resident bottom fauna) indicate a high level of correspondence. For instance, in Baltimore Harbor, Tsai et al. (1979) noted a significant correlation between sediment bioassay results for the tested sites.

In the case of acute lethal tests with the phoxocephalid amphipod, Rhepoxynius abronius, sediment bioassay results correspond closely with actual field distributions of phoxocephalids. Swartz et al. (1982) noted that phoxocephalid amphipods were absent from industrialized areas of Commencement Bay where sediment was acutely toxic in laboratory bioassays to R. abronius, but phoxocephalids were ubiquitous in areas where sediment was not toxic. Similar results were obtained in California, where Swartz et al. (in press b) found that the survival of R. abronius, in sewage-contaminated sediments from the Palos Verdes Shelf, was significantly correlated with the spatial distribution of phoxocephalids and the structure of benthic communities. Oakden et al. (1984) showed that phoxocephalids avoided contaminated sediment from the Palos Verdes Shelf.

Broad-scale comparisons of sediment bioassay results with overall sediment chemistry and bottom fish histopathology data have shown a direct correspondence in Puget Sound (Quinlan et al., in press). Also in Puget Sound, Chapman et al. (in press b) have shown that areas characterized as toxic in sediment bioassays are also characterized by changes in benthic infaunal composition and elevated overall sediment contaminant levels. Detailed studies of the Commencement Bay Superfund site in Puget Sound indicate a high level of correspondence between sediment bioassay results, bottom fish histopathology, benthic community structure, and overall sediment chemistry levels (Krull and Ginn, 1984).

Freshwater environment

Prater and Anderson (1977) found that the results of acute lethal sediment bioassays in an Ohio creek correlated well with in situ benthic communities. Malueg et al. (1984a, b) found similar correlations between sediment bioassay results and the field distributions of benthic macroinvertebrates.

USES OF SEDIMENT BIOASSAYS

Sediment bioassays can be used, both in marine and freshwater environments, for a number of purposes associated with assessment and regulation. First, they can be used to determine the relative toxicity of sediments in different areas to determine areas of maximum and/or unacceptable toxicity for remedial action. In this context sediment bioassays often serve as the initial step in a tiered approach to marine pollution assessment, which may ultimately include measures of both sediment chemistry and benthic community structure in the most toxic areas.

An example of how sediment bioassays can be used to determine highly toxic areas is provided in Figures 1 and 2. Combined data are presented from separate studies conducted in two of the Waterways of Commencement Bay, an area listed by the U.S. Environmental Protection Agency as among the top ten priority toxic waste dumps in the United States (Long, 1982). As noted by Swartz et al. (1982), there is a great deal of variability in toxicity at different sites, indicating that single station test results cannot be used to reliably represent the toxicity of entire areas. There are a number of areas (i.e. around Pennwalt in the Hylebos and at the Lincoln Avenue storm drain in both Waterways) that unquestionably contain a high proportion of toxic sediments based on positive results from more than one test. Because extrapolation from single samples can lead to erroneous conclusions depending on whether the samples are truly representative, Figure 2 presents a summary of the data in terms of broad-scale toxicity patterns related to more than one test and generally, depending on sample patterns, to several stations in an area.

A second way that sediment bioassays can be used is to determine any changes in toxicity after a management and/or engineering action such as the removal of a point source or treatment upgrading. This information will provide a direct measure of the effectiveness of management actions. Such use of sediment bioassays may be preceded by dilution studies (toxic sediment mixed with clean sediment) to determine the degree of change in the sediments necessary to eliminate the toxic response. Such dilution studies have been conducted off municipal sewage discharges in Puget Sound (Chapman and Fink, 1983) and in industrialized areas of Commencement Bay, (P. Chapman, unpub. data).

Third, sediment bioassays may be used as part of permitting programs to determine the acceptability of dredged material and other wastes for aquatic disposal. Such testing involves a comparison of the toxicity of the waste material with that of sediment from the disposal site. The U.S. EPA has recently authorized the Four-Mile Rock open-water dredge disposal site in Puget Sound on the basis that prospective dredged material must be tested (including bioassays) to ensure that it is not more toxic than the sediment presently found at the disposal site. The U.S. Army Corps of Engineers is similarly using sediment bioassay in the approval process for freshwater dredging and disposal (Leonard, 1983).

Finally, sediment bioassays may be used in laboratory studies of the relative toxicity of different components of sediment contaminant mixtures. For instance, Swartz et al. (1984) added sewage sludge from different treatment

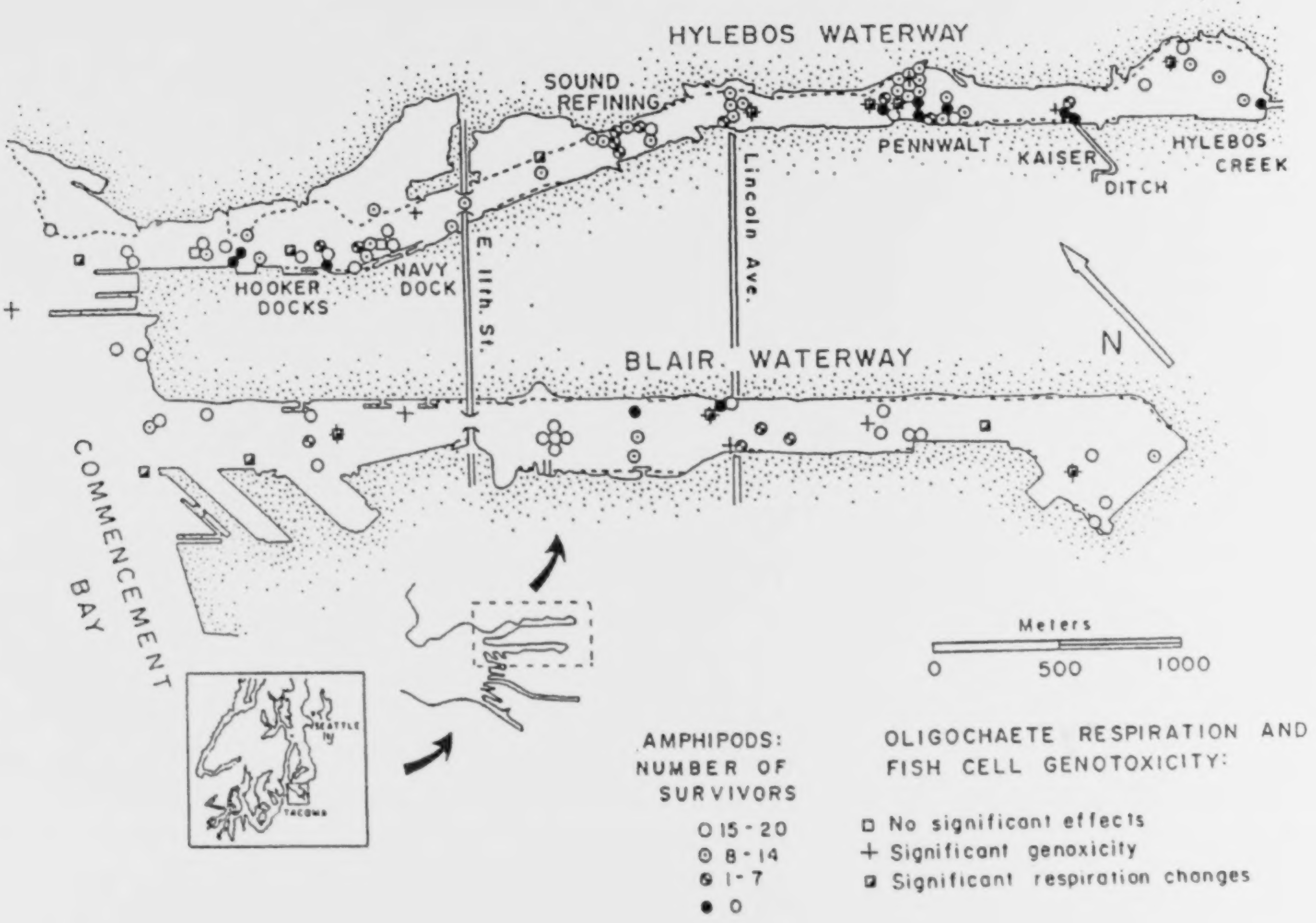
HYLEBOS WATERWAY
SOUND REFINING
PENNWALT
KAISER
DITCH
HYLEBOS CREEK
HOOKER DOCKS
NAVY DOCK
E. 11th St.
Lincoln Ave.
BLAIR WATERWAY
N
COMMENCEMENT BAY
SEATTLE
TACOMA
Meters
0
500
1000
AMPHIPODS: NUMBER OF SURVIVORS
○ 15 - 20
⊙ 8 - 14
⊗ 1 - 7
● 0
OLIGOCHAETE RESPIRATION AND FISH CELL GENOTOXICITY:
□ No significant effects
+ Significant genoxicity
▣ Significant respiration changes

Figure 1. Data on sediment toxicity testing in two Commencement Bay Waterways, U.S.A. Amphipod acute lethality data are from Swartz et al. (1982) where 100% survival = 20 amphipods; other data are from Chapman et al. (1982a).

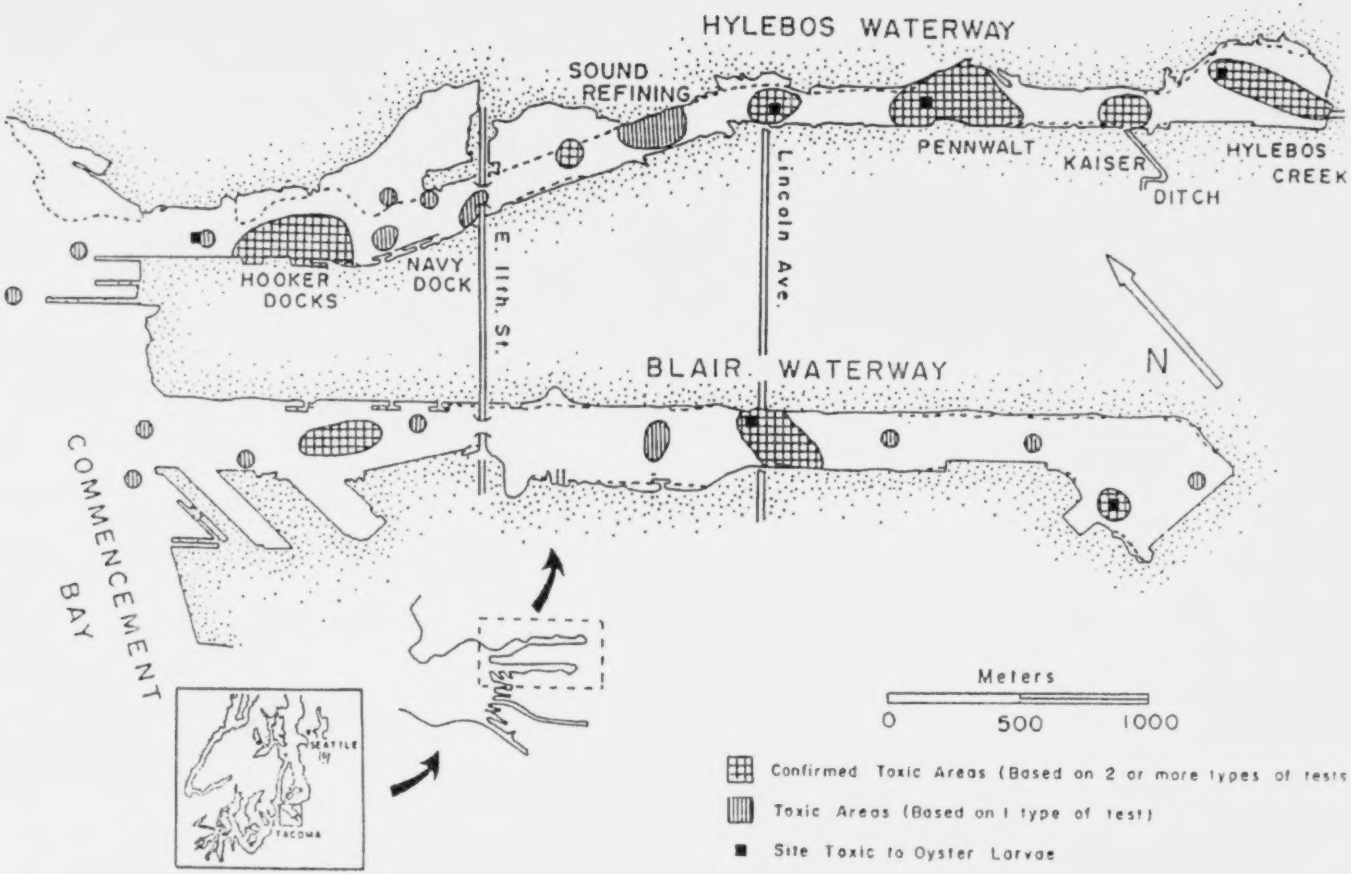
HYLEBOS WATERWAY
SOUND REFINING
PENNWALT
KAISER
DITCH
HYLEBOS CREEK
HOOKER DOCKS
NAVY DOCK
E 11th St.
Lincoln Ave.
BLAIR WATERWAY
N
COMMENCEMENT BAY
SEATTLE
TACOMA
Meters
0
500
1000
Confirmed Toxic Areas (Based on 2 or more types of tests
Toxic Areas (Based on 1 type of test)
Site Toxic to Oyster Larvae

Figure 2. Determination of toxic areas in two Commencement Bay Waterways based on data from Figure 1 and additional data. Oyster larvae bioassay data are from Chapman et al. (1983); all six sites tested in this latter study are shown. Confirmed toxic areas were first established, and then the oyster larvae data were overlaid.

plants to uncontaminated sediment and used sediment bioassays to obtain an estimate of the relative toxicity to the sludge from different sources. Schuytema et al. (1984) used Daphnia sediment bioassays to determine the availability of cadmium in spiked sediments, related to free ion levels. Cairns et al. (1984) used various freshwater bioassays with copper-spiked sediments as a first step in determining water quality criteria for copper in sediments. In addition, sediments can be fractionated and/or spiked to determine and rank toxic chemicals found in the sediments (Samoloff et al., 1983).

RELEVANCE OF SEDIMENT BIOASSAYS TO THE OCEAN DUMPING CONTROL ACT (ODCA)

In Canada, the ocean disposal of dredged spoils including contaminated sediments and other solid wastes is regulated by permit under the Ocean Dumping Control Act (ODCA), promulgated on December 13, 1975 as a result of international agreement under the 1972 London Dumping Convention. Under the ODCA maximum sediment quantities and concentrations for a number of potentially hazardous compounds (e.g. cadmium and mercury) were established. As part of the permit granting process, the ODCA also stipulated additional factors which should be taken into account, including: "accumulations and biotransformations in biological materials or sediments". However, no guidelines or test protocols were established for assessing the biological accumulation of sediment-associated contaminants.

As a result, the Canadian Environmental Protections Service (EPS) has, over the last few years, commissioned a large number of studies into the bioaccumulation of selected metals (in particular Cd and Hg) and organic compounds (PCBs) from sediments by aquatic organisms. Despite considerable research efforts, a useful bioaccumulation protocol for testing has not been established (cf. Haywood et al., 1983, McGreer et al., 1984). In addition, present evidence indicates that bioaccumulation of metals from sediments is not of concern in the marine environment (e.g. mercury - McGreer et al., 1983; cadmium - Taylor, 1984). Although there is substantial evidence that bioaccumulation of some metals occurs leading to relatively high residue levels in tissues of certain species, this phenomenon appears to be restricted to a few species and it appears likely that these residues reflect complexation products of a detoxification process (Taylor, 1984).

Although organic compounds such as PCBs may be bioaccumulated and biomagnified up food chains, the majority of organic compounds accumulated by aquatic organisms are metabolized such that bioaccumulation studies show no evidence of uptake (Quinlan et al., in press). However, metabolic products can induce adverse biological effects such as histopathological disorders and tumors (Malins et al., 1984).

Even when high levels of particular chemicals are detected in the tissues of organisms, the relevance of these findings to the health of the affected organisms (i.e. "So what?") is extremely difficult to establish. Moreover, bioaccumulation studies tend to be very expensive and time-consuming.

In contrast, established sediment bioassay test protocols provide cost-effective and rapid data (bioassays can be completed within 2 to 10 d) on the toxicity of complex sediment contaminant mixtures. Death as an end-point in acute lethal tests provides compelling and convincing evidence for government agencies, managers, and the lay public, while sublethal effects determined in more sensitive tests provide more subtle indications of possible ecological effects. The lack of toxicity in sediments tested by a range of bioassay techniques provides similarily convincing evidence that this material will not cause adverse ecological effects in the aquatic environment. Consequently, these tests are more relevant to permitting under the ODCA than previous bioaccumulation studies.

RECOMMENDED SEDIMENT BIOASSAY TECHNIQUES

Because different biological tests do not necessarily respond to the same toxicant(s) (Salmoloff et al., 1983), it is recommended that sediment bioassay testing incorporate more than one end-point. Ideally, and as outlined by Chapman and Long (1983), sediment bioassay testing should incorporate acute lethal, sublethal and genotoxic responses. Effectively, this means that three separate tests should be conducted on each sediment tested.

Marine environment

Acute lethal testing in the marine environment should be conducted using the 10-d Rhepoxynius abronius test described by Swartz et al. (1982, in press a). This test basically consists of exposing 20 amphipods in each of the 5 replicate containers to a 2 cm layer of sediment in full 1 L beakers and determining survival after 10 d. The amphipods typically burrow into the sediments, and emergence may be used as a measure of sublethal effects.

The R. abronius test is widely used by the U.S. EPA as an initial screening tool, and consistent results have recently been determined in interlaboratory calibration tests. Five laboratories tested seven different sediments comprising samples from four different field sites and three samples spiked with different concentrations of cadmium. There was good agreement among laboratories concerning the relative and absolute toxicity of the test sediments (A. Mearns, U.S. NOAA, pers. comm.).

Sublethal testing may be conducted using either the oyster larvae bioassay (Chapman and Morgan, 1983), or the oligochaete respiration test (Chapman, 1984). The oyster larvae test consists of exposing developing, fertilized eggs to sediment elutriates and determining both survival and abnormalities after 48 h. The test is extremely sensitive but, because oyster spawning is difficult to induce during the winter months, cannot reliably be used all year. The oligochaete respiration test consists of exposing worms to sediment elutriates and determining any significant abnormalities in their respiration rate over a 4 to 6 h period. This test is slightly less sensitive than the oyster larvae test, but can be conducted year-round.

Genotoxicity testing should be conducted using the anaphase aberration test developed by Kocan et al. (1982). This test consists of exposing cultured rainbow trout gonad cells to sediment extracts for 72 h during the active growing phase and determining the number of cells that undergo chromosomal damage during the anaphase stage of mitosis. The test is extremely sensitive and, although it appears to respond to natural as well as anthropogenic compounds in sediments, presently comprises the best available means for sediment genotoxicity testing.

Freshwater environment

Acute lethal testing in the freshwater environment should be conducted using the 48 h Daphnia magna test described by Nebeker et al. (1984a.) This test consists of exposing waterfleas to sediments in a similar beaker system to that described for the marine amphipod R. abronius. The test is uncomplicated and sensitive.

Sublethal test methods for the freshwater environment are in the development stages, with most work being performed by the U.S. EPA in Corvallis, Oregon (G. Chapman, U.S. EPA, pers. comm.). Presently it appears that a 10 d D. magna life-cycle test may be the most appropriate to use (Nebeker et al., 1984). Testing is conducted in replicate with 20 5-d old D. magna exposed in 2.5 L of water to 500 ml of test sediment; survival and reproductive success are assessed after 10 d (three broods).

Genotoxicity testing has not been broadly conducted in freshwater sediments, however the genotoxicity test described for marine sediments has been successfully used in limited freshwater testing (E.V.S. Consultants, unpub. data). This test is recommended for freshwater applications.

SUMMARY

Sediments provide a cumulative sink for the majority of persistent aquatic contaminants. Sediment bioassays provide a rapid and reproducible measure of in situ toxicity to resident populations, such that direct linkages can be made between particular contaminated areas and biological effects. A positive (or negative) sediment bioassay response eliminates guesswork and speculation regarding the toxicity of sediments.

In the marine environment of Puget Sound, areas determined to be of highest sediment toxicity in laboratory bioassays corresponded with those in which in situ biological disorders were manifest (i.e., benthic infaunal community changes, hepatic lesions in fish and crustaceans). In freshwater environments similar relationships have been observed.

Chemical measurements of contaminants in sediments provide little information on sediment toxicity because: [1] thousands of chemicals are known to be toxic but not all of these are quantifiable; [2] toxic responses can be caused by different types of chemicals in different sites/areas; and [3] toxicity is rarely due to any single chemical, being more likely due to mixtures of chemicals. The bioassay response is to overall sediment contamination, and as noted by Swartz (1984): "the sediment bioassay is a method of pollution assessment whose validity is not dependent on correlation with sediment chemistry".

It is inappropriate that present Canadian regulations dealing with identification and disposal of contaminated sediments rely on chemical analyses alone. As discussed in this paper, sediment bioassay tests provide toxicity data that are necessary for assessment and regulation. Sediment bioassays should be incorporated into such legislation as the Ocean Dumping Control Act to provide a sound foundation for environmental management.

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THE INFLUENCE OF PH ON THE ACUTE LETHALITY OF FENITROTHION, 2,4-D AND AMINOCARB AND SOME PH ALTERED SUBLETHAL EFFECTS OF AMINOCARB TO RAINBOW TROUT

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Many factors can influence the toxic effects of pesticides. Since acid precipitation is becoming an increasing problem in Eastern Canada, it was decided to determine the effects of pH on the acute lethality of three pesticides widely used in this area: 2,4-dichlorophenoxyacetic acid (2,4-D); aminocarb; and fenitrothion.

Technical grade pesticides were tested in static, 96-hour acute lethal toxicity bioassays to determine the LC_{50} of each pesticide, at varying ambient pH levels, to fingerling rainbow trout (*Salmo gairdneri*). The mean test pH values were 4.5, 5.5, 7.0 and 8.5. The toxicity of 2,4-D decreased with increasing pH with LC_{50}'s ranging from 67.3 mg/L at pH 4.5 to >1000 mg/L at pH 8.5. In the case of aminocarb, the reverse relationship was observed with LC_{50}'s ranging from 106.0 mg/L at pH 4.5 to 7.5 mg/L at pH 8.5. The toxicity of fenitrothion did not vary substantially within the pH range tested.

A subsequent experiment tested the effect of variable exposure pH (4.5 and 8.5) on the aminocarb uptake and brain acetylcholinesterase activity of rainbow trout. In the fish tested at the lower pH, whole body concentrations of aminocarb reached a maximum at 6 hours and declined until 96 hours at which time all exposed fish were alive. In the fish tested at the higher pH the concentration of pesticide remained elevated until all exposed fish were dead. Likewise in the fish exposed to the pesticide at the lower pH maximum brain acetylcholinesterase depression was observed at 6 hours after which it returned to normal levels. In the fish exposed at the higher pH brain acetylcholinesterase activity was progressively depressed up to the point of death.

Possible mechanisms to explain these results are discussed.

L'INFLUENCE DU PH SUR LA LÉTALITÉ AIGUE DUE AU FÉNITROTHION, 2,4-D ET AU CARBAMINE, ET QUELQUES EFFETS SOUS-LÉTAUX DU CARBAMINE CHEZ LA TRUITE ARC-EN-CIEL SOUS PH ALTÉRÉ

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Plusieurs facteurs peuvent influencer les effets toxiques des pesticides. Depuis que les précipitations acides sont devenues un problème de plus en plus pressant dans l'est du Canada, on a décidé de déterminer les effets du pH sur la létalité aigue causée par trois pesticides largement utilisés dans cette région: l'acide 2,4-dichlorophénoxyacétique (2,4-D), le carbamine et le fénitrothion.

Des pesticides calibrés furent testés au moyen de bioassais statiques de toxicité létale aigue de 96 heures, en vue de déterminer le CL_{50} de chaque pesticide à différents niveaux de pH, pour des alevins de truites arc-en-ciel (Salmo gairdneri). Les valeurs moyennes de pH des analyses étaient de 4.5, 5.5, 7.0 et 8.5. La toxicité du 2,4-D diminua avec des pH de plus en plus élevés, avec des CL_{50} variant de 67.3 mg/L à un pH de 4.5, à >1000 mg/L à un pH de 8.5. Dans le cas du carbamine, la relation inverse fut observée, avec un CL_{50} variant de 106 mg/L à pH de 4.5 à 7.5 mg/L à pH de 8.5. La toxicité du fénitrothion ne varia pas substantiellement dans les variations de pH analysées.

Une expérience subséquente testait les effets de variations de pH (4.5 et 8.5) sur l'assimilation du carbamine et sur l'activité de l'acétylcholinestérase dans le cerveau de la truite arc-en-ciel. Chez les poissons analysés à des pH bas, la concentration du carbamine dans tout le corps atteignait un maximum après 6 heures et diminuait jusqu'à 96 heures, alors que tous les poissons étaient encore vivants. Chez les poissons testés a de hauts pHs, la concentration du pesticide demeurait élevée jusqu'à ce que tous les poissons meurent. Similairement, les poissons exposés au pesticide au plus bas pH démontraient une diminution extrême dans le niveau d'acétylcholinestérase dans le cerveau après 6 heures, après quoi le niveau remontait à un état normal. Chez les poissons exposés au pH élevé, l'activité de l'acétylcholinestérase dans le cerveau diminuait progressivement jusqu'au point où la mort s'ensuivait.

Des méchanismes possibles pour expliquer ces résultats sont discutés.

EXTENDED ABSTRACT

Many factors can influence the toxic effects of pesticides. Since acid precipitation is a problem in Eastern Canada, it was decided to determine the effects of pH on the acute lethal toxicity of three pesticides widely used in this area: 2,4-dichlorophenoxyacetic acid (2,4-D); aminocarb and fenitrothion.

Technical grade pesticides were tested in static, aerated, 96 h acute lethal toxicity tests using fingerling rainbow trout (Salmo gairdneri Richardson). Each pesticide was tested at pH 4.5, 5.5, 7 and 8.5. The toxicity of 2,4-D, an acid, ranged from 67 mg/L at pH 4.5 to 420 mg/L at pH 5.5, 1,000 mg/L at pH 7 and >1,000 mg/L at pH 8.4, a remarkable decrease in toxicity with increasing pH. For aminocarb, a base, the toxicity ranged from 107 mg/L at pH 4.5 to 61 mg/L at pH 5.5, 54-57 mg/L at pH 7 and 7.5 mg/L at pH 8.5, a notable increase in toxicity with increasing pH. For fenitrothion, which does not ionize at the pH range tested, the toxicity did not change significantly with changing pH advanced from 1.9 to 3.3 mg/L. These observations are, in general, in agreement with the pH partition theory, but it was found that degree of ionization did not totally account for the change in toxicity of a compound, especially at the higher pH's.

A follow-up experiment was conducted to test the effect of pH on brain acetylcholinesterase (AChE) activity and aminocarb uptake by rainbow trout. Fish were exposed to 10 mg/L aminocarb at pH 4.5 and pH 8.5. For the first 6 hours exposure, aminocarb levels in fish tissues were similar at both pH's, ranging from 6.2 to 12 µg/g. After six hours, however, aminocarb level in pH 4.5 exposed fish declined to 1 to 2 µg/g and remained low until the end of the test while in pH 8.5 exposed fish they remained elevated until all fish had died by 72 hours. Brain AChE activity in the fish showed an inverse relationship to aminocarb levels in fish tissue. At pH 4.5, brain AChE activity dropped to 58% of that of control fish by 6 hours, and increased to levels similar to those for control fish by the end of the test. At pH 8.5, however, brain AChE activity dropped to 40% of that of control fish by 6 hours, and remained depressed until all fish had died by 72 hours.

Aminocarb is 6.6% undissociated at pH 4.5 and 99.9% undissociated at pH 8.5. Thus, according to the pH partition theory, we would expect greater than ten times more aminocarb to enter fish tissues at the higher pH. This was the case at 24 hours exposure and beyond, but for the first 6 hours of the experiment aminocarb uptake was similar at both pH's. Various possible mechanisms are discussed, but at present we have no conclusive explanation for the unexpectedly large tissue residues at pH 4.5 for the first 6 hours of exposure. However, their effects were marked, depressing brain AChE activity and causing the fish to become sluggish. Only when tissue residues declined at pH 4.5 (after 24 hours) did these sublethal effects begin to disappear.

SELECTED ORGANIC CONTAMINANTS IN FISH AND SEDIMENTS FROM THE FRASER RIVER ESTUARY

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A survey was made of selected organic contaminants in fish tissue and surface sediments from 10 sites in the Fraser River Estuary of British Columbia. Starry flounder tissue contained high levels of several chlorinated phenols with tetrachlorophenol from 0.19 to 2.52 µg/g and pentachlorophenol from 0.77 to 2.77 µg/g wet weight. Bioconcentration of the 6 chlorinated phenols increased in proportion to the number of chlorine atoms substituted on the phenol which is directly related to the octanol/water partition coefficient of these compounds. Phenol was the only acidic organic contaminant found in sediments and concentrations were lower than levels found in fish tissue. The phthalates that occurred most frequently in both fish tissue and sediments were diethyl-, di-n-butyl- and the bis (2-ethylhexyl-) esters. Benzylbutylphthalate was only found in fish tissue. Polycyclic aromatic hydrocarbons (PAH's) occurred more frequently in sediments than in fish tissue. Phenanthrene, fluoranthene and pyrene were the most common PAH's in the base/neutal extracts of sediments while naphthalene, fluoranthene and benzo(a)pyrene occurred more often in fish.

The highest levels of chlorinated phenols were found in fish collected from a slough adjacent to a major landfill site and from the Annacis Island and Iona Island sites which are near the two major sewage outfall areas in the Fraser River Estuary. The highest concentrations of PAH's and phthalate esters in sediments were also found adjacent to the landfill site, the sewage treatment plant near Iona Island and in the north arm of the river which receives direct industrial discharges and urban runoff.

CONTAMINANTS ORGANIQUES DANS LES POISSONS ET SÉDIMENTS DE LA RIVIÈRE FRASER

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Une étude de certains agents contaminants organiques dans les tissus de poissons et dans les sédiments de surface fut conduite en 10 endroits dans l'estuaire de la rivière Fraser de la Colombie Britannique. Les tissus de plies tachetées contenaient de hauts niveaux de plusieurs phénols chlorés, avec le têtrachlorophénol ayant des valeurs allant de 0.19 à 2.52 µg/g et le pentachlorophénol variant de 0.77 à 2.77 µg/g de poids humide. La concentration des 6 phénols chlorés dans les tissus augmentait en proportion du nombre d'atomes de chlore substitués sur le phénol qui est directement lié au coefficient de partition octanol/eau de ces composés. Le phénol était le seul contaminant organique acide trouvé dans les sédiments, et ses concentrations en étaient plus basses que celles trouvées dans les tissus de poissons. Les phthalates apparaissant le plus souvent dans les tissus de poissons et dans les sédiments étaient le diéthyle-, di-n-butyle- et les esters bis (2-éthylhexyle). Le benzylbutylphthalate était seulement trouvé dans les tissus de poissons. Les hydrocarbures aromatiques polycycliques (HAPs) apparaissaient plus fréquemment dans les sédiments que dans les tissus de poissons. Le phénanthrène, fluoranthène et pyrène étaient les HPAs les plus communs dans les extraits basiques/neutres de sédiments, alors que le naphtalène, fluoranthène et benzo-(a)pyrène apparaissaient plus souvent chez les poissons.

Les plus hauts niveaux de phénols chlorés furent trouvés dans les poissons collectés dans un marécage adjacent à un majeur endroit de remblayage et provenant des îles Annacis et Iona, qui sont près de décharges majeures de rebuts domestiques dans l'estuaire de la rivière Fraser. Les plus hautes concentrations de HAPs et d'esters de phthalates dans les sédiments furent aussi trouvées près de l'endroit de remblayage, à l'usine de traitement des eaux usées près de l'île Iona et dans le bras nord de la rivière qui reçoit directement les décharges industrielles et les écoulements urbains.

INTRODUCTION

Many trace organic contaminants are very insoluble in water and adsorb readily to sediments. For persistent organic contaminants, the sediments often serve as a historical record of environmental contamination if they are deposited in undisturbed areas. The sediment characteristics (particle size, organic content, biological activity) are important factors in regulating the adsorption, degradation, and exchange reactions that occur between sediments and contaminants. Aquatic organisms are also good indicators of environmental contamination from organic pollutants. The relatively low solubility of many organic contaminants favours a partitioning into the fatty tissues of aquatic organisms (Veith et al., 1980; Kenaga and Goring, 1980). Bioaccumulation of persistent trace organic compounds can also take place as materials are concentrated through trophic levels of the food web (Metcalfe et al., 1971).

Recent studies to develop a management plan for the Fraser River Estuary have revealed scattered and incomplete information on the levels of organic contaminants in the estuary (Garrett, 1980). The objective of this study was to investigate selected trace organic contaminants associated with surface sediments and fish tissue at 10 stations in the Fraser River Estuary.

METHODOLOGY

Study area and sample collection

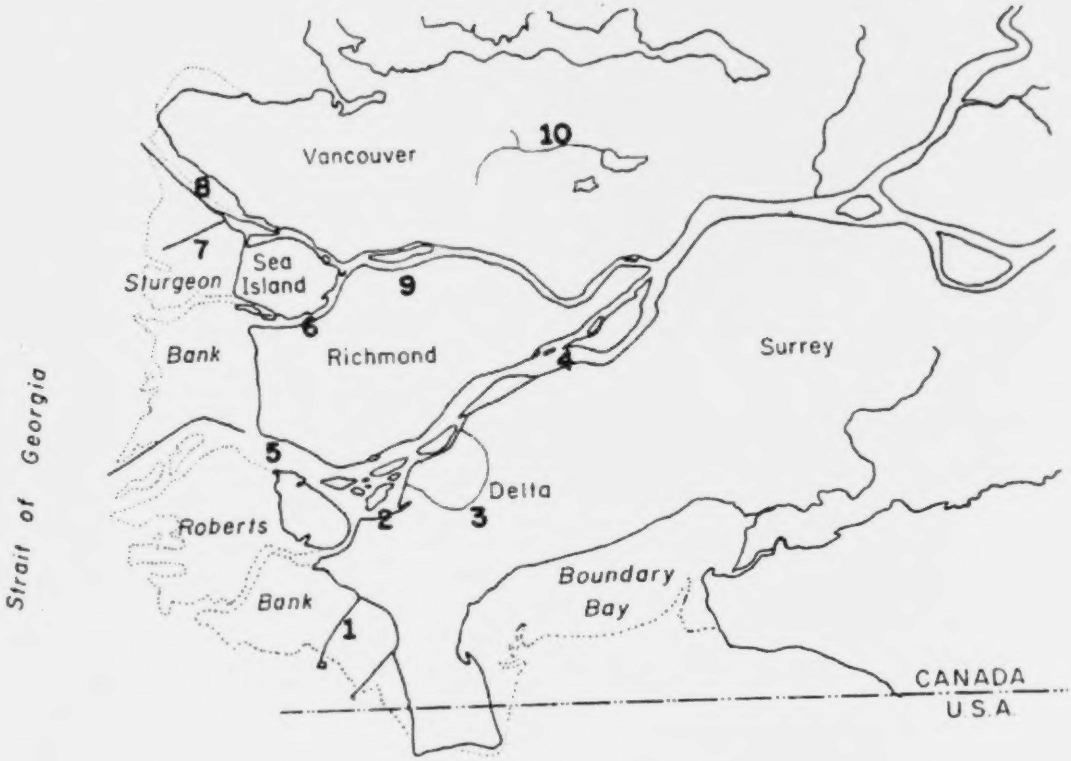
Ten sites were selected to represent a cross section of the estuarine environment that receives impacts from a variety of land use and water related activities. These sites included Roberts Bank [1] in the vicinity of a coalport terminal, Ladner Slough [2] a backwater harbour area, Crescent Slough [3] a freshwater ditch adjacent Burns Bog landfill, Annacis Island [4] adjacent to the large sewage treatment plant, Steveston Island [5] near the mouth of the Main Arm, Middle Arm [6] south-east of Sea Island, Iona Island [7] in the outfall area of the sewage treatment plant, Point Grey [8] adjacent to a large log booming area, North Arm [9] an area of high industrial activity, and Still Creek [10] an urban creek that receives stormwater runoff (Figure 1).

A series of five surface sediment samples was collected with a Ponar dredge at each site and placed in solvent rinsed wide mouth jars which were covered with aluminum foil, capped and placed in a cooler. In the laboratory a composite sample was prepared and submitted for extraction. Particle size analysis and organic matter determination were made to characterize the composite samples. Fish were collected at each site with a 50 meter beach seine. Individual fish were wrapped in aluminum foil and placed in a cooler. Fish were identified, measured (length, weight) and frozen until needed for analysis. Epaxial muscle tissue was carefully dissected for trace organic analysis unless the fish were too small in which case (i.e. Station 3 and 9) the heads were removed and the rest of the fish homogenized for analysis. Otoliths were dissected from selected starry flounders for age determination (Campana and Neilsen, 1982). All samples were collected in November and December 1983.

Sediment extraction and analysis

Fifty grams of wet sediment was diluted and the pH adjusted to 11 with 5N

Figure 1. Sampling sites in the Fraser River estuary.


10
Vancouver
8
7
Sea Island
Sturgeon
9
6
Bank
Richmond
4
Surrey
Strait of Georgia
5
Delta
2
3
Roberts
Boundary
Bank
Bay
1
CANADA
U.S.A.

NaOH. The aqueous slurry was extracted three times with 100 ml portions of dichloromethane by stirring the phases together in an Erlenmeyer flask for periods of one hour. The combined solvent extracts were dried over anhydrous Na_2SO_4, concentrated to 20 ml on a rotary evaporator and to one ml on a micro Kuderna-Danish apparatus to give the base-neutral fraction. The pH of the remaining sediment slurry was adjusted to <2 with 18N H_2SO_4, extracted three times with dichloromethane and concentrated as above to one ml to give the acid fraction. Absorption chromatography on silica gel, using a series of solvents of increasing polarity, was used to clean up the extracts if they were very dirty. Gas chromatography was performed on a Hewlett-Packard 5880A capillary gas chromatograph with a flame ionization detector and a DB-5 30m fused silica column.

Fish extraction and analysis

A monophase system of wet fish tissue (8-10 g), adjusted to pH 12 with NaOH, and a dichloromethane:methanol solvent mixture (2:1, 80 ml) was homogenized for 2 minutes and centrifuged. The extraction was repeated three times. The combined extract was concentrated by vacuum evaporation and further partitioned between water and dichloromethane. The dichloromethane extract was dried over anhydrous Na_2SO_4, isooctane was added as a carrier, and the solvents evaporated to a small volume to give the base-neutral fraction. The residual fish tissue was adjusted to pH 2 with 6N HCl and homogenized with 100 ml of dichloromethane:methanol (1:2) in a monophase system three times. The combined extracts were concentrated and cleaned up similar to the base-neutral fraction. Both extracts were subjected to gel permeation chromatography (BioBeads SX-3 resin on a Autoprep 102 Analytical Labs system) to remove high molecular weight fats and oils which could interfere with the gas chromatography. Gas chromatography was performed on a Varian-Vista Series 401 capillary gas chromatograph with a flame ionization detector and a SE-54 15 m fused silica column. For more details of the extraction techniques see the technical report (Hall et al. 1984).

The fat content of the fish tissue was determined by a monophase solvent extraction procedure (Bligh and Dyer, 1959).

Gas chromatography/mass spectroscopy (GC/MS)

Confirmation of compounds tentatively identified by gas chromatographic retention times was made on a Hewlett Packard 5985 GS/MS equipped with a computerized library data system for spectral comparison. The sensitivity and mass assignment was checked with d8 naphthalene as an internal standard. The compounds selected for identification and quantification were taken from a list of EPA priority pollutants (Table 1). In some cases, trace amounts of a compound were identified by GC/MS but could not be quantitated by gas chromatography. The actual detection level for the traces reported will depend upon the specific compound. The GC/MS detection level for polycyclic aromatic hydrocarbons (PAH's) was 0.5 ng, for phenols, chlorinated benzenes and phthalates 1.0 ng, for high boiling PAH's 5.0 ng and for nitrophenols 250 ng.

RESULTS

Quality control

To assess the possibility of laboratory contamination, especially for phthalates, blank controls were run for sediments and fish tissue. These blank controls involved carrying solvents and reagents through the extraction and concentration procedures to see if they contained any residual contaminants. The chromatographic profiles of these blanks demonstrated no significant contamination problem.

Sample splits were analyzed for organic contaminants to look at the reproducibility of the techniques. There were differences in the identification of some peaks from their retention times, especially for chlorobenzenes. This emphasizes the importance of GC/MS analysis for positive identification. Quantitative differences were certainly apparent in sample splits. This is probably attributable to three factors. First, it is often difficult to take identical sub-samples from a heterogenous sediment mixture. Secondly, the multiplication factors used to convert the peak heights from µl amounts to injected sample to the total sample extract can magnify small differences in peak heights. Thirdly, variable recoveries from spiked samples could produce differences observed in sample split analysis.

The recovery from spiked sediment samples varied with different compounds. There appeared to be very poor recovery of the high molecular weight PAH's (ca. 10-30%) from sediments. Recovery of most of the acidic phenols from sediments was usually less than 50 percent. Although recovery studies from fish tissues were not as comprehensive as for sediments, due to more time required to develop an extraction technique, one detailed experiment showed good recovery of chlorobenzenes (71-100%), most phthalates (82-103%) and lower molecular weight PAH's (70-108%). Recovery of the lower molecular weight phenols was poor (19-39%) probably due to loss of these more volatile compounds during evaporation of the extract. Recovery of the higher chlorinated phenols was better (60-96%).

Organic contaminants in sediments

The organic contaminants found in the ten composite sediment samples are presented in Table 2. The phthalate esters, mainly diethyl-, di-n-butyl- and bis(2-ethylhexyl)- and the lower molecular weight PAH's (naphthalene to chrysene) were the most common organic contaminants found in the base/neutral sediment extract. The phthalates varied from a trace to 595 ng/g while the PAH's varied from a trace to 335 ng/g dry weight. Phenol was the only acidic contaminant found in sediments from 7-56 µg/g dry weight. The highest concentrations of phthalates were found in the highly organic (12%) Cresent Slough sediment. The Point Grey and North Arm stations contained some of the highest levels of PAH's.

In addition to the composite sediment sample, two separate samples collected close to the Iona Island sewage treatment plant outfall were analyzed for trace organics (Table 3). These separate samples contained higher numbers of phthalates and PAH's than the composite sample made up of samples collected from 0.5 to 2.5 km from the outfall. The higher concentrations of phthalates were in the sample closest to the outfall while the 1.0 km sample contained higher concentrations of PAH's. These preliminary results could reflect the association of contaminants with different particle size materials from the wastewaters since there is a gradual decrease in organic matter and increase in particle size of sediments with distance from the outfall.

Organic contaminants in fish tissue

Tissue from starry flounder (Platichthys stellatus) was analyzed from all stations except for station 3 (Crescent Slough) where peamouth chub (Mylocheilus caurinus) were analyzed. For the peamouth chub at Crescent Slough and the small starry flounders collected in the North Arm station the whole fish (minus head) was homogenized for analysis while for the larger starry flounder, which varied in age from 1-4 years (10-380 g) only the epaxial muscle tissue was dissected for analysis.

Diethylphthalate and bis(2-ethylhexyl)phthalate were found in all fish and varied in concentration from a trace to over 1,000 ng/g. Dibutylphthalate was found in 7 out of 9 fish and benzylbutylphthate in 5 out of 9 fish so the phthalate esters appear to be fairly widely distributed in the Fraser River Estuary fish (Table 4). The PAH's were less prevelant in fish than sediments. Naphthalene was the most commonly occurring PAH (found in 7 out of 9 fish) followed by benzo(a)pyrene and fluoranthene both found in only three fish. The peamouth chub from Crescent Slough and the large starry flounder (377 g) from Steveston Island contained the largest number of PAH's.

Phenol and several chlorinated phenols were identified in fish tissue (Table 5). Pentachlorophenol and tetrachlorophenol (mainly the 2,3,4,5-isomer) occurred in concentrations between 194-2700 ng/g wet weight in 8 out of 9 fish samples analysed. With the exception of the fish from Roberts Bank, 2,4-dichlorophenol and 2,4,6-trichlorophenol were found in all fish; however concentrations were usually lower than for the higher chlorinated phenols. Phenol was found in all fish and varied from 4-320 ng/g wet weight.

DISCUSSION

Quality control

The poor and variable recovery of some groups of organic contaminants from sediments and fish tissue means that only approximate estimates of some compounds can be made. Other investigators have also obtained poor recoveries from complex matrices such as sediment and sewage sludge (Lopez-Avila et al., 1981). The dilemma that researchers on trace organics face is that even recovery studies, where solvent solutions of standards are spiked to the environmental sample, do not give reliable estimates of the ability of the technique to recover contaminants that are more firmly bound to a complex environmental matrix such as sediment or tissue. An attempt was made to overcome this problem for the fish tissue extraction where solvent ratios were carefully selected to give a monophase mixture which should give much better substrate-solvent contact during the homogenization process.

Many investigators make derivatives of the acidic organic determinants fraction to achieve better extraction and facilitate the chromatography (Lin et al., 1979). However, the rates of formation of methylated phenolic compounds varies considerably and some compounds begin to degrade before the formation of others is complete. Since analytical sensitivity and chromatographic separation were acceptable for the acidic components, derivatization was not conducted during this study.

Organic contaminants in sediments

Pyrene, fluoranthene and phenanthrene, which are PAH's of intermediate molecular weight, were the most common PAH's found in Fraser Estuary sediments. The highest concentration of PAH's were found in sediments collected from the North Arm of the river (North Arm and Point Gray stations). This area of the river receives many direct industrial discharges and stormwater runoff from the Vancouver area. Polycyclic aromatic hydrocarbons are well known contaminants in exhaust particulates and used motor oil (NRC, 1983) therefore the most likely source of these materials is urban runoff. Although the composite sediment sample taken from the Iona Island sewage treatment plant outfall area only contained a high concentration of pyrene, the 1.0 km sample contained several PAH's. The sewage system which services this treatment plant collects both wastewater and stormwaters therefore urban runoff is probably an important source of PAH's in the outfall area of the sewage treatment plant.

A comparison of selected PAH's found in the Fraser River Estuary and other areas near Vancouver is made in Table 6. Burrard Inlet, which includes the Vancouver Harbour area and False Creek, a poorly flushed marine inlet off English Bay, both contained much higher levels of PAH's than were found in the Fraser. This is attributable to much better flushing of the Fraser with its annual freshet, and a much higher level of urbanization for a longer period of time in the Vancouver core area adjacent to Burrard Inlet and False Creek.

The phthalate esters are used extensively as plasticizers (Morita et al., 1974). These high boiling esters appear to be quite stable and are found throughout the environment although their acute toxicity is much lower than organic contaminants such as pesticides. The highest levels of phthalates were found in sediments from Crescent Slough which is adjacent to the Burns Bog sanitary landfill site. Leachates from this landfill, which contain a lot of plastic material, are the most likely source of phthalate contamination of sediments from this area.

The marine environment adjacent to metropolitan Vancouver (False Creek and Burrard Inlet) also contained much higher levels of phthalates than was found in the Fraser River Estuary (Table 6). Again, this is attributable to the much poorer flushing of this area.

Organic contaminants in fish tissue

With the exception of naphthalene, PAH's were only found in four samples of fish tissue collected at 9 stations in the Fraser River Estuary. Rogers (1979) found that selected PAH's ranged from 1-112 ng/g wet weight which is within the range of values (trace-302 ng/g) found in fish during this study. The identification of benzo(a)pyrene in three samples of starry flounder tissue (27-115 ng/g) could be important since it is a well known carcinogen. Dunn and Stich (1976) have found benzo(a)pyrene in tissues from marine organisms and suggest that it could be related to the proximity of the organisms to creosoted pilings.

Phthalate esters were widely distributed in fish analyzed from the Fraser River Estuary. There was no apparent relationship between the phthalates in the fish, their size or area of collection. Other investigators (Rogers, 1979 - starry flounders from Sturgeon Bank, and Singleton, 1983 - largescale suckers from Main and North Arms of the river) have identified phthalate esters within the range of concentrations found in this study.

The relative concentrations of the chlorinated phenols found in fish from the different stations showed a general increase as the number of chlorine atoms substituted on the phenyl ring increase. As the number of chlorine atoms on the molecule increases the octanol/water partition coefficient increases (Lin et al., 1982). This means that compounds with more chlorine would prefer to diffuse through the lipid membrane of a living cell rather than remain in the hydrophilic environment outside the cell. However, the concentration of these compounds found in organisms can be affected by the levels of pollutants discharged to the aquatic environment and the rate of microbial degradation in the environment as well as characteristics of the molecules.

The Fraser River Estuary, especially the North Arm, is highly dominated by the forest products industry which makes extensive use of wood preservatives containing chlorinated phenols. Tetrachlorophenol and pentachlorophenol are two of the main ingredients in this preservative and were found in the highest levels in fish (see Table 5). If this wood preservative is the main source of the chlorinated phenols found in fish, one would expect higher levels to be found in fish from the North Arm of the river (Point Grey and North Arm stations) but this was not found during our investigation. The highest concentrations were found in fish collected from Crescent Slough adjacent to the sanitary landfill and in fish collected near the outfall areas of the sewage treatment plants (Iona Island and Annacis Islnd stations). Leachates from building demolition materials at the landfill could contribute to chlorinated phenols to Crescent Slough. The direct discharge of phenolic wastes to the sewerage system by the forest product industries might explain higher levels in fish collected near treatment plant outfalls.

The levels of chlorinated phenols found in starry flounders during this investigation and in largescale suckers analyzed by Singleton (1983) are compared in Table 7. The starry flounders contained higher levels of all the chlorinated phenols than were found in suckers. These differences could reflect differences in analytical techniques or in the feeding behaviour and habitat preference of the two fish. Both fish tend to be bottom dwellers. However, chlorinated phenols do not appear to accumulate in sediments since only phenol was found in the acidic sediment extracts and it is known that microorganisms can degrade phenols (Kirsch and Etzel, 1973; Tabek et al., 1964). Therefore the fish must accumulate the chlorinated phenols through direct absorption from the water or from their feeding habits. It will require a more detailed investigation to identify the extent of the chlorinated phenol problem in the Fraser River Estuary.

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Table 1. Trace organic contaminants determined in sediments and fish tissue.

A. Acidic Fraction

- phenol
- 2,4-dimethylphenol
- 2-nitrophenol
- 4-nitrophenol
- 2,4-dinitrophenol
- 4,6-dinitro-o-cresol
- 4-chloro-3-methylphenol
- 2-chlorophenol
- 2,4-dichlorophenol
- 2,4,6-trichlorophenol
- 2,3,5,6-tetrachlorophenol
- 2,3,4,5-tetrachlorophenol
- 2,3,4,6-tetrachlorophenol
- pentachlorophenol

B. Base-Neutral Fraction

- Chlorobenzenes
  - 1,3-dichlorobenzene
  - 1,4-dichlorobenzene
  - 1,2-dichlorobenzene
  - 1,2,4-trichlorobenzene
  - hexachlorobenzene
- Phthalates
  - dimethylphthalate
  - diethylphthalate
  - di-n-butylphthalate
  - bis(2-ethylhexyl)phthalate
  - benzylbutylphthalate
  - di-n-octylphthalate
- Miscellaneous
  - nitrobenzene
  - isophorone
  - hexachlorobutadiene
- Polycyclic Aromatic Hydrocarbons
  - naphthalene
  - 2-chloronaphthalene
  - acenaphthylene
  - acenaphthene
  - fluorene
  - phenanthrene
  - anthracene
  - fluoranthene
  - pyrene
  - benzo(a)anthracene
  - chrysene
  - benzo(b)fluoranthene
  - benzo(k)fluoranthene
  - benzo(a)pyrene
  - indeno(1,2,3,-c,d)pyrene
  - dibenz(a,h)anthracene
  - benzo(g,h,i)perylene

Table 2. Trace organic contaminants in sediments[1].

Compound	Sampling Station[2]									
	1	2	3	4	5	6	7	8	9	10
Phthalates										
Diethyl-	+		160	+		+	+	+		+
Di-n-butyl-	+	+	300		105	220	446	+	+	64
Bis(2-ethylhexyl)-		50	595		21	+	170	+	+	57
Butylbenzyl-								196		
PAH's										
Naphthalene			+			+	+	+	+	
Acenaphthene		+		+		+				
Fluorene			98	+				+		
Phenanthrene	+	26	46	18	13	24	+	75	32	35
Anthracene							+	3		
Fluoranthene	+	61	+	38	23	79	+	68	139	53
Pyrene	+	102	+	89	43	174	311	258	335	108
Benzo(a)anthracene		6				+		+	18	
Chrysene		+				4		+	+	
Acidic Compounds										
Phenol	56		28	7	8		13	28	9	25

[1] all values in ng/g dry wt., + = trace.

[2] Sampling Stations;

1 - Roberts Bank
2 - Ladner Harbour
3 - Crescent Slough
4 - Annacis Island
5 - Steveston Island
6 - Middle Arm
7 - Iona Island
8 - Point Grey
9 - North Arm
10 - Still Creek

Table 3. Organic contaminants in sediment from Iona Island[1].

Compound	0.5 km	Sampling Site[2] 1.0 km	0.5-2.5 km (Composite)
Phthalates			
Dimethyl-	+		
Diethyl-	190	102	+
Di-n-butyl-	204	60	446
Bis(2-ethylhexyl)	844	404	170
Benzylbutyl-	+	+	
Di-n-octyl-	94	+	
PAH's			
Naphthalene	+	20	+
Acenaphthylene	+	+	+
Acenaphthene		53	
Fluorene		45	
Phenanthrene	44	62	+
Anthracene		154	
Fluoranthene	115	173	+
Pyrene	45	71	311
Benzo(a)anthracene		78	
Chrysene		32	

[1] all values in ng/g dry wt., + = trace.
[2] distance from sewage treatment plant outfall.

Table 4. Base neutral organic contaminants in fish[1].

Compound	Sampling Station[2]								
	1	2	3	4	5	6	7	8	9
Chlorobenzenes									
1,4-Dichloro-		32							21
1,2-Dichloro-		6		101					
Phthalates									
Dimethyl-			14						74
Diethyl	72	+	51	284	313	124	237	136	257
Di-n-butyl-	73	15			518	+	622	128	242
Bis-(2-ethylhexyl)	8	27	24	25	93	1057	588	19	156
Benzylbutyl-			14	316	170			139	100
PAH's									
Naphthalene	43	+	34	+	109			8	19
Fluorene			12						
Phenanthrene			10		3				
Anthracene					143				
Fluoranthene			5		18				204
Pyrene			17						8
Chrysene			8		302				
Benzo(a)pyrene			27		135			36	
Benzo(g,h,i)perylene			58		+				

[1] all values in ng/g wet wt., + = trace.

[2] Sampling Stations;

1 - Roberts Bank	4 - Annacis Island	7 - Iona Island
2 - Ladner Harbour	5 - Steveston Island	9 - Point Grey
3 - Crescent Slough	6 - Middle Arm	10 - North Arm

Table 5. Acidic organic contaminants in fish[1].

Compound	Sampling Station[2]								
	1	2	3	4	5	6	7	8	9
Phenol	49	12	320	112	15	4	105	9	33
2-Chlorophenol			123		6		40	3	13
2,4-Dichlorophenol		337	182	736	41	6	217	94	74
2,4,6-Trichlorophenol		519	365	1442	493	357	602	547	57
Tetrachlorophenol		1908	2522	1732	1562	194	2084	587	952
Pentachlorophenol		1140	2768	2329	2182	773	2111	1093	933

[1] all values in ng/g wet wt., + = trace.

[2] Sampling Stations;

1 - Roberts Bank
2 - Ladner Harbour
3 - Crescent Slough
4 - Annacis Island
5 - Steveston Island
6 - Middle Arm
7 - Iona Island
8 - Point Grey
9 - North Arm

Table 6. Comparison of selected base/neutral contaminants in surface sediments[1].

Compound	Sediment Source		
	Fraser Estuary	False[2] Creek	Burrard[2] Inlet
Di-n-butylphthalate	64	200	240
Bis(2-ethylhexyl)phthalate	35	1770	770
Naphthalene	+	460	420
Phenanthrene	25	920	2140
Fluoranthene	46	1670	2290
Pyrene	105	3470	2680
Benzo(a)anthracene		890	530

[1] median values in ng/g dry weight, + = trace. Fraser Estuary n = 10, False Creek and Burrard Inlet n = 9.

[2] Hall et al. (1983).

Table 7. Comparison of organic contaminants in Fraser estuary fish tissue[1].

Compound	Starry[2] Flounders	Largescale[3] Suckers
Trichlorophenol	506 (<3-1442)	<20 (<20-60)
Tetrachlorophenol	1257 (<3-2522)	<10 (<10-250)
Pentachlorophenol	1116 (<3-2768)	35 (<10-190)
Benzybutylphthalate	54 (<8-316)	38 (29-54)

[1] all values in ng/g wet weight, median values with range in brackets.
[2] n = 8 this investigation.
[3] n = 10, Singleton (1983), from estuary stations.

EFFECTS OF WOOD WASTE ON THE RECRUITMENT POTENTIAL OF MARINE BENTHIC COMMUNITIES

Kathman, R.D.[1], S.F. Cross[2] and M. Waldichuk.[3] 1984.

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Replicate samplers containing varying concentrations of wood wastes and sediments were deployed in 25 m of water in outer Burrard Inlet on 1 August 1983 for benthic invertebrate colonization. After 11 weeks, on 20 October 1983, the samplers were retrieved and the benthic macroinvertebrates were identified and enumerated. Data analyses included dominance and diversity measures, hierarchical (cluster) classification and ordination techniques. Species richness, diversity and evenness values were highest and dominance values were lowest in the 20% wood waste samples, compared to the 0%, 50% and 100% wood waste concentrations. The sample cluster analysis clearly differentiated two groups: one containing almost all of the 0% and 20% samples and the other containing almost all of the 50% and all of the 100% samples. Species cluster analysis indicated three distinct groups of taxa. The first group, comprised of polychaetes and oligochaetes, and the second group, containing polychaetes and bivalves, are both indicative of low concentrations of wood fibres. The third group contained nematodes, the wood-burrowing shipworm *Bankia setacea*, and the polychaetes *Armandia brevis*, *Capitella capitata* and *Prionospio cirrifera*, all typically associated with high levels of organic enrichment (pollution). The similar patterns among all data analyses confirmed the greater recruitment potential of marine macroinvertebrates in sediments containing some wood wastes. Enhancement occurred between 10% and 40% wood content, while higher wood content was detrimental to marine organisms.

LES EFFETS DES DÉCHÊTS LIGNEUX SUR LE POTENTIEL DE RECRUTEMENT DE COMMUNAUTÉS MARINES BENTHIQUES

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Des substrats de colonisation pour invertébrés benthiques contenant des concentrations variées de déchets ligneux et de sédiments, furent déployés dans 25 m d'eau à l'extérieur de Burrard Inlet le 1er août 1983. Aprés 11 semaines, le 20 octobre 1983, les substrats furent récupérés et les macroinvertébrés benthiques furent identifiés et énumérés. L'analyse de ces données comprenait des mesures de dominance et de diversité, une classification hiérarchique (en grappes) et des techniques d'ordination. Comparés aux échantillons avec des concentrations de déchets ligneux de 0, 50 et 100%, les échantillons à 20% avaient les plus hautes valeurs en nombre, en diversité d'espèces et en coefficient d'uniformité et avaient aussi les valeurs les plus basses en termes de dominance. L'analyse en grappes des échantillons distinque clairement deux groupes: un contenant presque tous les échantillons à 0 et à 20%, et un autre contenant presque tous les échantillons à 50% et tous ceux à 100%. L'analyse en grappes des espèces indique trois groupes distincts de taxons. Le premier groupe comprend des polychètes et des oligochètes, et le deuxième comprend des polychètes et des bivalves. Ces deux groupes sont indicateurs de basses concentrations en fibres de bois. Le troisième groupe contient des nématodes, le taret *Bankia setacea*, et les polychètes *Armandia brevis*, *Capitella capitata* et *Prionospio cirrifera*, tous typiquement associés à de hauts niveaux d'enrichissement organique (pollution). La similarité de toutes les analyses de données confirme le grand potentiel de recrutement de macroinvertébrés des sédiments contenant des déchets ligneux. Un accroissement du recrutement eut lieu entre 10 et 40% de contenu ligneux, tandis qu'un plus haut contenu ligneux s'avéra nuisible aux organismes marins.

INTRODUCTION

Many studies have been conducted relating occurrence, distribution and diversity of marine benthic invertebrates to contaminants and organic enrichment (see, for example, Dean and Haskin, 1964; Kathman et al., 1983; Pearson, 1972 and 1975; Thom and Chew, 1984; and others). Few studies, however, have dealt with in situ experiments determining colonization and succession of the benthos (see, for example, Arntz and Rumohr, 1982; Bonsdorff, 1980; Pearson and Rosenberg, 1978), and none of these has dealt specifically with the effects of wood wastes and fibres on the potential recruitment and maintenance of benthic invertebrates, although Conlan (1977) conducted an intensive quantitative survey of marine infaunal and epifaunal invertebrates associated with a log handling site at Mill Bay, B.C. Observations made from the submersible "Pisces IV" have shown a depauperate benthic fauna associated with sediments containing a high wood fibre content and other contaminants (Hoos, 1977; Packman, 1980), but no quantitative infaunal surveys were conducted.

The present study represents initial experiments to investigate the effects of varying concentrations of wood wastes in sediments on the colonization potential of benthic invertebrates. Long-term studies are needed to delineate specific factors (exposure time, depth of wood waste) influencing recolonization and specific effects (species presence, abundance and survival) of different concentrations of wood wastes.

TERMS OF REFERENCE

The overall objective of this study was to determine the effects of different concentrations of wood waste on the recruitment of marine benthic invertebrates. Specific objectives were:

[1] placement of in situ samplers containing 0, 20, 50 and 100 percent wood wastes to allow recruitment of benthic macroinvertebrates;
[2] retrieval of samplers with the colonizing benthos;
[3] quantification and identification of benthic macroinvertebrates found in each sampler;
[4] analyses of substrate particle size, total organic carbon and total nitrogen;
[5] statistical analyses of these data to determine whether any significant differences exist among different concentrations of wood wastes; and,
[6] preparation of a report discussing the findings of the study.

MATERIALS AND METHODS

Benthic invertebrates

Sediments were collected by divers at 15-20 m depths near the West Vancouver Laboratory dock facilities in outer Burrard Inlet, B.C. The sediments were frozen (-20°C) for several days to kill existing fauna, thawed, homogenized and mixed with wood wastes. Wood wastes (wood fibres and wood by-products) were collected from a loading dock area in the intertidal zone at Port Mellon in Howe Sound, B.C. Seasoned, pulverized wood chips exposed to water and wave action were used to simulate typical wood-rich material found near dumpsites and to reduce problems associated with soluble extractives (e.g. sugars, humic acids, tannins and lignin) in newly-processed wood. Wood wastes were added to natural sediments in volumetrically-determined proportions (V/V). Ratios of wood wastes to natural sediments were 0:100, 20:80, 50:50 and 100:0. Each mixture was thoroughly homogenized and placed into two replicate wooden containers as shown in Figure 1.

The containers were securely fastened to a large wooden base and the entire device was anchored by divers in outer Burrard Inlet at a bottom depth of approximately 25 m, near the original collection site of the sediment. The containers were retrieved after 11 weeks (01 August - 20 October) exposure. Each container was securely covered with a lid, brought to the surface, and transported to the E.V.S. Consultants laboratory within two hours. In the laboratory, a specially constructed divider-grid was inserted into each container, and the four middle subsections (to avoid any 'edge' effects as discussed in Berge (1980)) were individually removed, providing a total of eight subsamples for each wood waste concentration (see Fig. 1). Each subsample was placed into a labelled plastic bag and preserved with 7-10% formalin, for taxonomic analysis. The remaining sediment from one container of each treatment was analyzed by Pacific Soils Ltd. for sediment particle size, total nitrogen and total organic carbon. Total organic carbon was determined by the Wakley-Black wet oxidation method. Total nitrogen was determined colorimetrically on a sulfuric acid digest, using modified micro Kjeldahl procedures. The pipette method as outlined by Walton (1978) was used for particle size analysis.

For taxonomic analysis, each sample was washed in a 0.5 mm mesh sieve to remove excess formalin and fine sediment. Contents remaining in the sieve were placed into a plastic container and enough water added to cover all material. Small aliquots of the sample were placed into a gridded petri dish and examined under a Wild MSA stereomicroscope. All benthic invertebrates were removed, enumerated by major taxonomic categories (generally Class or Order) and placed into 60 percent isopropanol. This process was continued until the entire sample had been examined and all organisms removed. Where sample volume was excessive, samples were volumetrically divided into two portions, one portion sorted for benthic invertebrates and the other archived. Detailed identification to the lowest possible taxonomic level consistent with presently available literature was performed for all organisms. A list of taxonomic references used for the identifications is provided in Appendix A.

Data analyses

Initial examination of the matrix of species abundances in response to varying wood fibre concentrations indicated that the proposed parametric statistical approach should be replaced by a descriptive methodology, employing recognized community classification techniques. High variability in abundances of taxa between replicates under any one treatment (wood fibre concentration) prompted consideration of each replicate separately, rather than as single treatment samples represented by average abundances of occurring taxa. High variability, in itself, was an important result of this experiment and was maintained, where possible, throughout the subsequent analyses.

Matrix Editing: The original data matrix (M1) was visually inspected for taxa which could be regarded as rare or incidental. These forms were removed

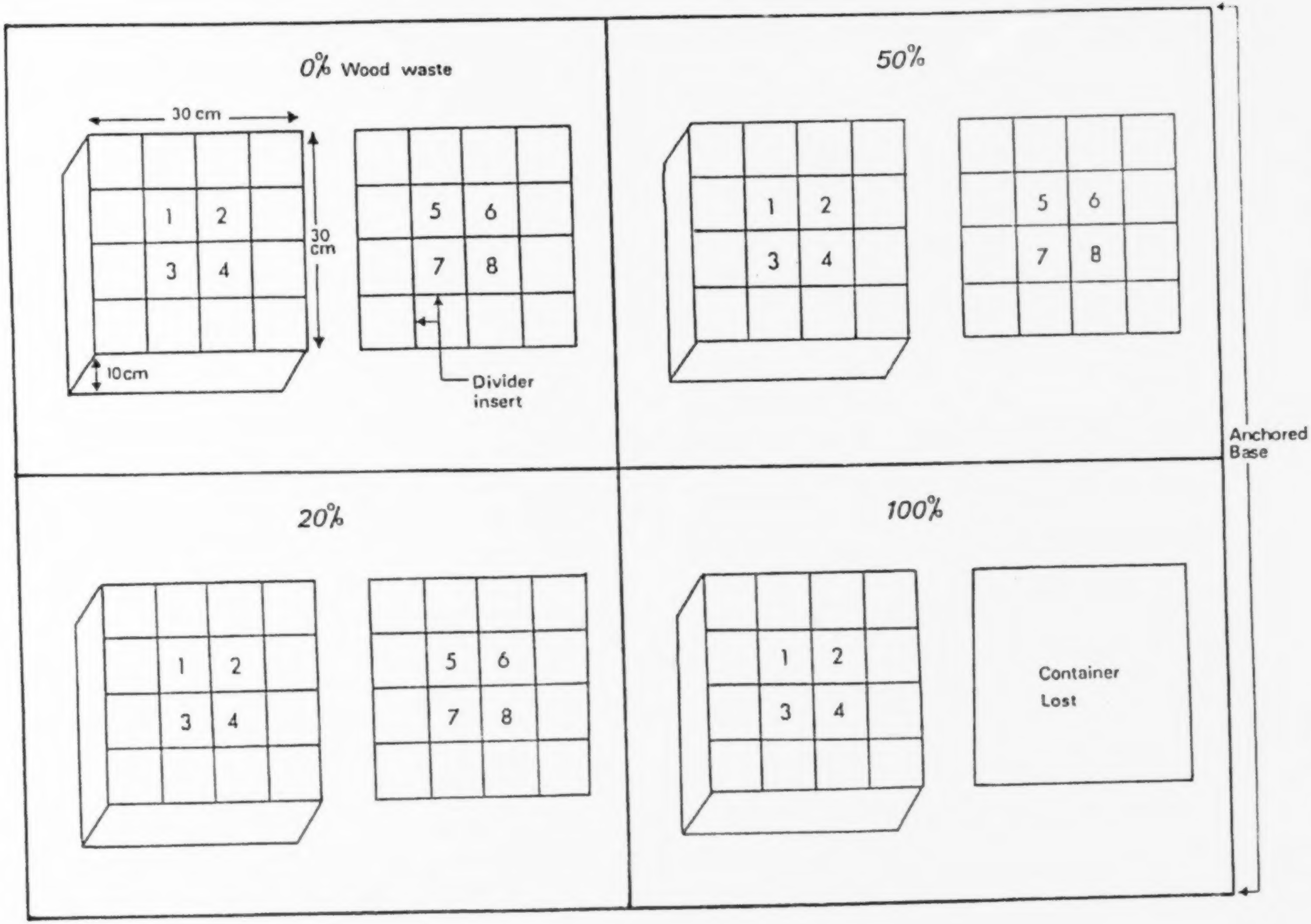
0% Wood waste
30 cm
30 cm
10cm
1
2
3
4
5
6
7
8
Divider insert
50%
20%
100%
Container Lost
Anchored Base

Figure 1. Base with attached samplers for benthic invertebrate recruitment study.

from the original matrix using two editing criteria:

i)	First edit	• deletion of taxa with recorded abundances of one or less in approximately 95% (26 of 28) of the treatment samples. Resultant matrix designation M2; and
ii)	Second edit	• deletion of taxa with recorded abundances of two or less in approximately 95% (26 of 28) of the treatment samples. Resultant matrix designation M3.

Each matrix (M1-M3) was subjected to the following analyses to allow comparison between matrices, and to thus reveal differences which may have resulted from the editing procedure.

Diversity/dominance measures

The species composition and abundance data for each of the 28 samples comprising the three data matrices were compared on the basis of species richness (s), and the Shannon-Weaver (1963) diversity index:

$$[1] \qquad H' = -\sum p_i \log p_i$$

where p_i = the abundance of species i.

Additional measurements included Pielou's (1966) evenness index:

$$[2] \qquad J' = \frac{H'}{H'_{max}}$$

where $H'_{max} = \log (s)$

a dominance measure, expressed as the complement of evenness (I - J'), and Simpson's (1949) diversity index:

$$[3] \qquad d = \frac{\sum n_i(n_i-1)}{N(N-1)}$$

where n_i = the number of individuals in the i^{th} species, and
N = the total number of individuals in the sample.

Mean diversity and dominance values were graphically displayed as a function of percent wood fibre. General trends observed in these relationships were compared among data matrices M1, M2 and M3.

Cluster analysis

Each data matrix was subjected to two hierarchical (cluster) analyses in order to allow comparison of:

i) samples, based on the similarity of species composition and abundances (Q-type analysis), and

ii) species, based on the similarity of occurrence in samples and their respective abundances (R-type analysis).

The complement of the Bray-Curtis coefficient was employed as the index of similarity in all trials, and is defined as:

$$C = 1 - [2w \div (a+b)] \quad [4]$$

where w = the sum of the lesser abundances for each species common to a pair of samples (in Q-type analysis), and

$(a+b)$ = the sum of abundances for each sample under comparison.

Pair-group clustering was unweighted (arithmetic means) and output was displayed as an optimally rotated dendrogram. These analyses were performed using the FORTRAN program "FAUNA I" developed by E.M. Hagmeier at the University of Victoria (© 1983).

RESULTS

A listing of all benthic invertebrates collected during this study is provided in Appendix B, and distributional data are provided in Appendix C. Homogeneous invasion and distribution within each container was assumed, allowing treatment of the subsamples as eight replicates of each concentration. One of the two containers with 100% wood wastes was lost prior to retrieval, providing only four replicates for this concentration.

Total and mean numbers of taxa were distinctly higher at 20% wood fibre than at the other concentrations (Fig. 2), and total numbers of organisms were similar betwen 0% and 50%, and between 20% and 100% wood fibre. A more detailed analysis (Table 1) shows large variations for most of the major taxonomic groups, especially the polychaetes and bivalve mulluscs. Although the wood fibre 100% treatment had the fewest species of polychaetes, the number of individuals was almost twice that of the next highest abundance, almost entirely due to large numbers of *Capitella capitata* and *Armandia brevis* in the 100% samples. Bivalves showed a similar pattern, with equally high numbers of individuals at all concentrations, but a significant decrease in diversity at 100%, where *Bankia setacea* was heavily dominant.

Species richness (= number of taxa), Shannon-Weaver diversity index, evenness, dominance and Simpson diversity index data are presented in Appendix D and summarized in Figure 3. These values showed a similar pattern to that described above among concentrations for all matrices. Species richness increased at 20% but showed a dramatic reduction at 100%. Diversity and evenness were highest at 20%, with slight decreases at 0% and 50%, and a large decrease at 100%. Dominance, the reciprocal of evenness, indicated that only a few species represented the majority of individuals at the 100% treatment, but that there were no particular species dominant at the other three concentrations.

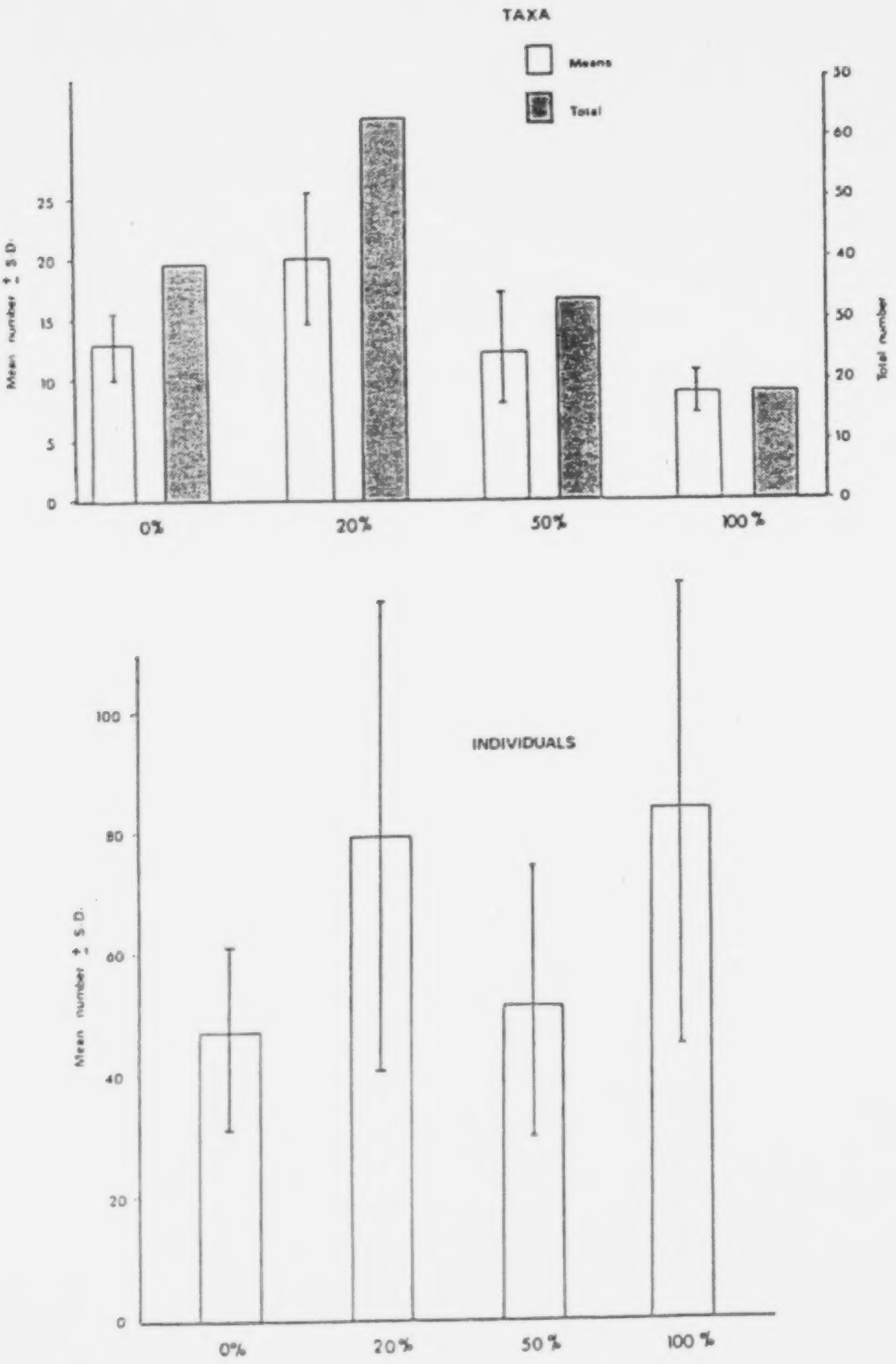

Figure 2. Numbers of taxa and numbers of individuals in original data matrix (28 samples x 81 taxa).

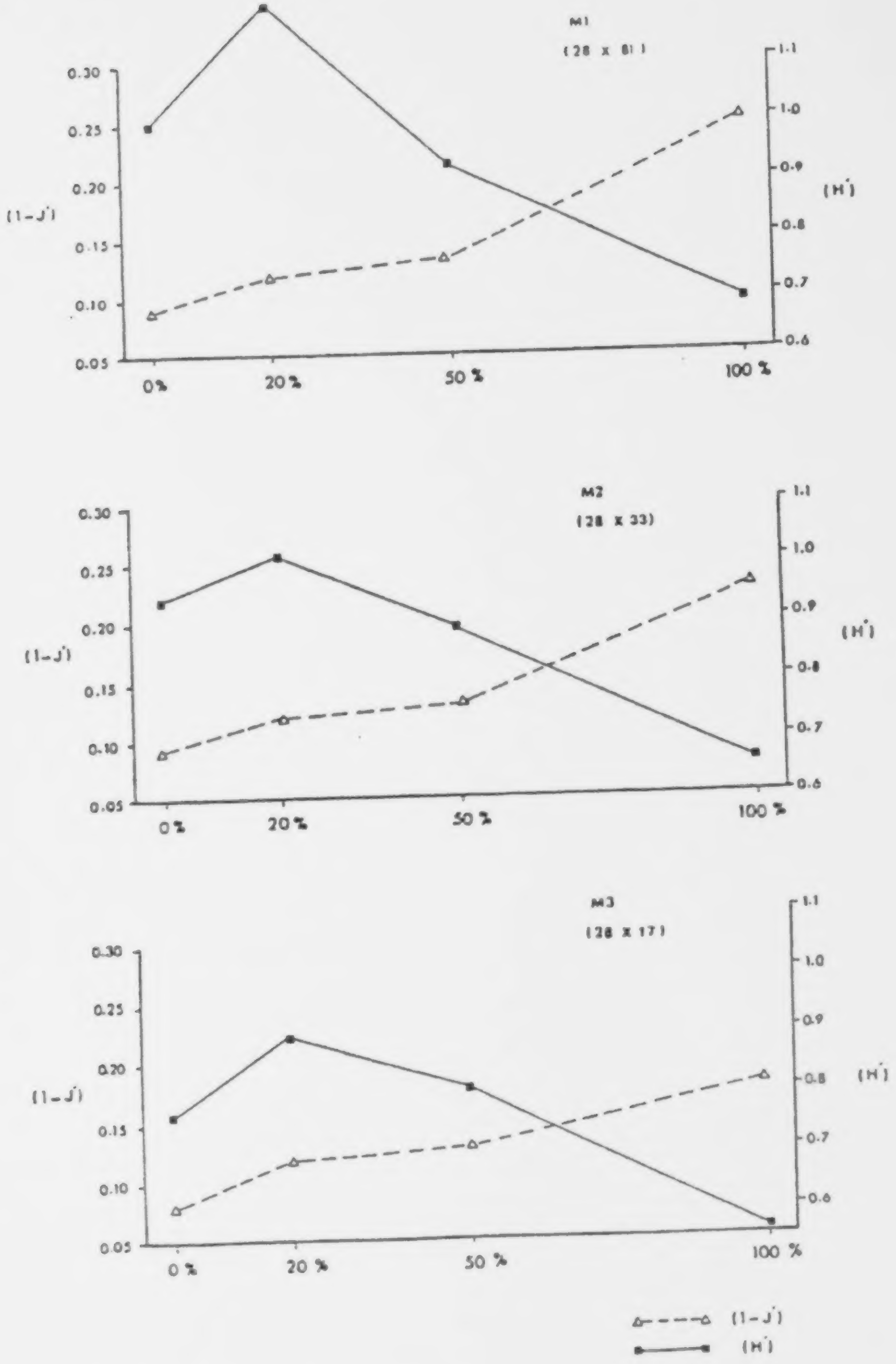

Figure 3. Trends in mean diversity (H') and mean dominance (I-J') for the three data matrices analyzed.

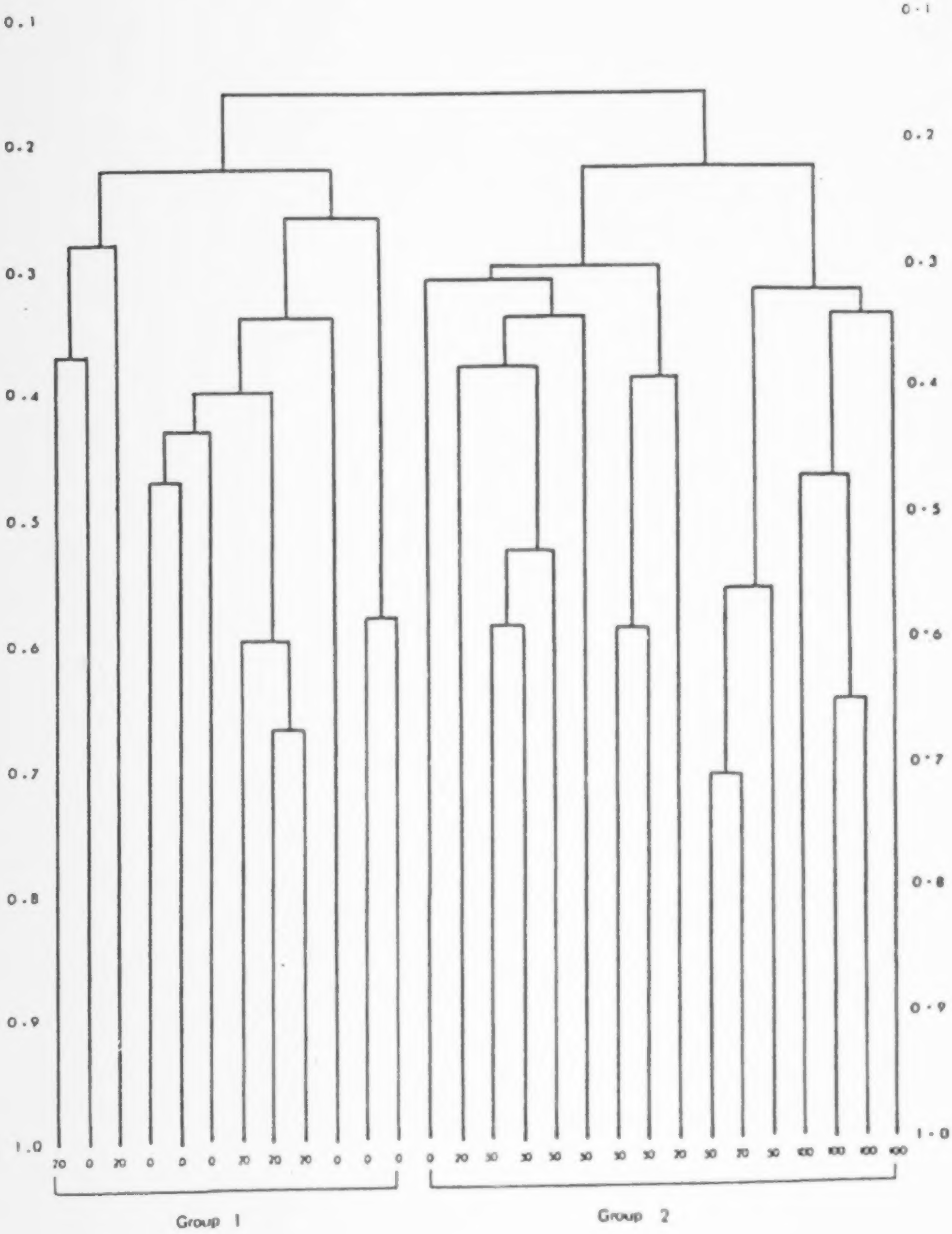

Figure 4. Cluster analysis treatment groupings derived from M1 (original) data matrix.

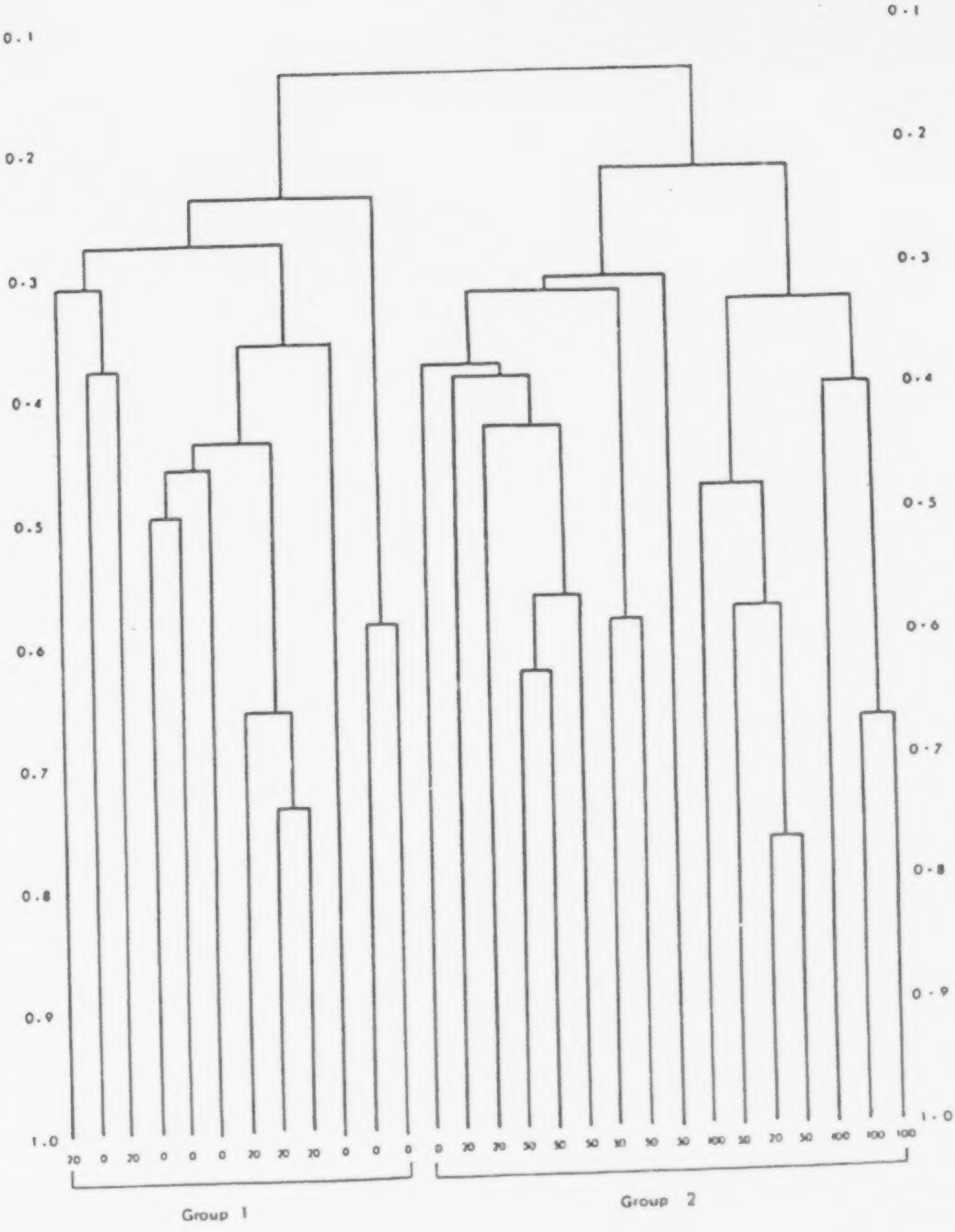

Figure 5. Cluster analysis treatment groupings derived from M2 (first edit) data matrix.

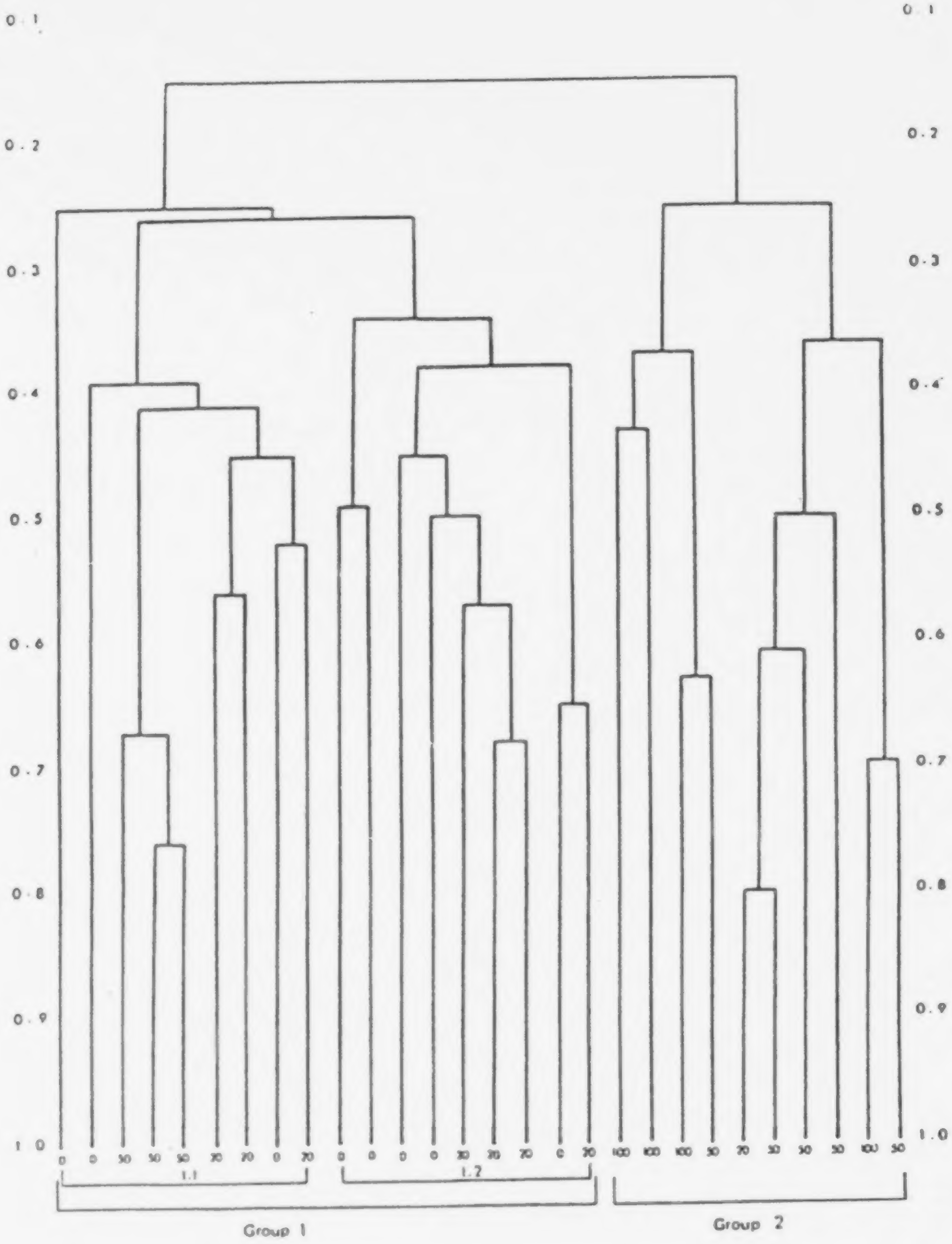

Figure 6. Cluster analysis treatment groupings derived from M3 (second edit) data matrix.

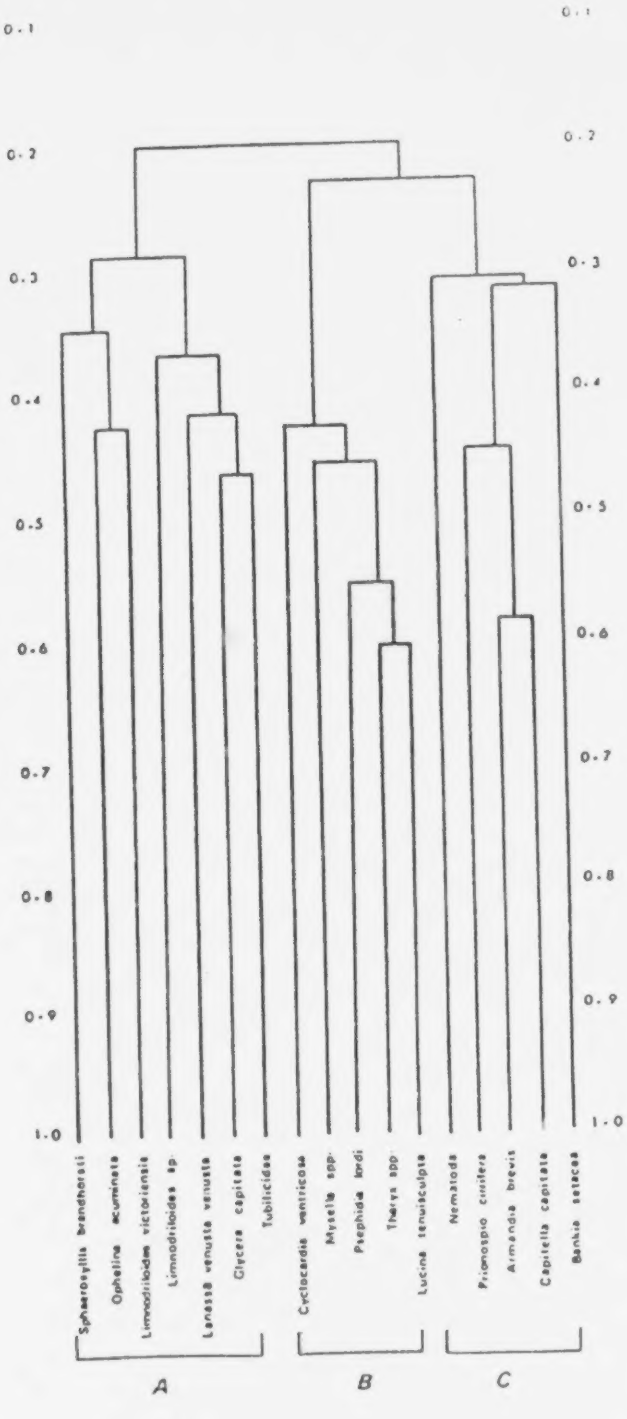
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
0.9
1.0
Sphaerosyllis brandhorsti
Ophelina acuminata
Limnodriloides victoriensis
Limnodriloides sp.
Lanassa venusta venusta
Glycera capitata
Tubificidae
Cyclocardia ventricosa
Mysella spp.
Psephidia lordi
Tharyx spp.
Lucina tenuisculpta
Nematoda
Prionospio cirrifera
Armandia brevis
Capitella capitata
Bankia setacea
A
B
C

Figure 7. Cluster analysis species groupings derived from M3 (second edit) data matrix.

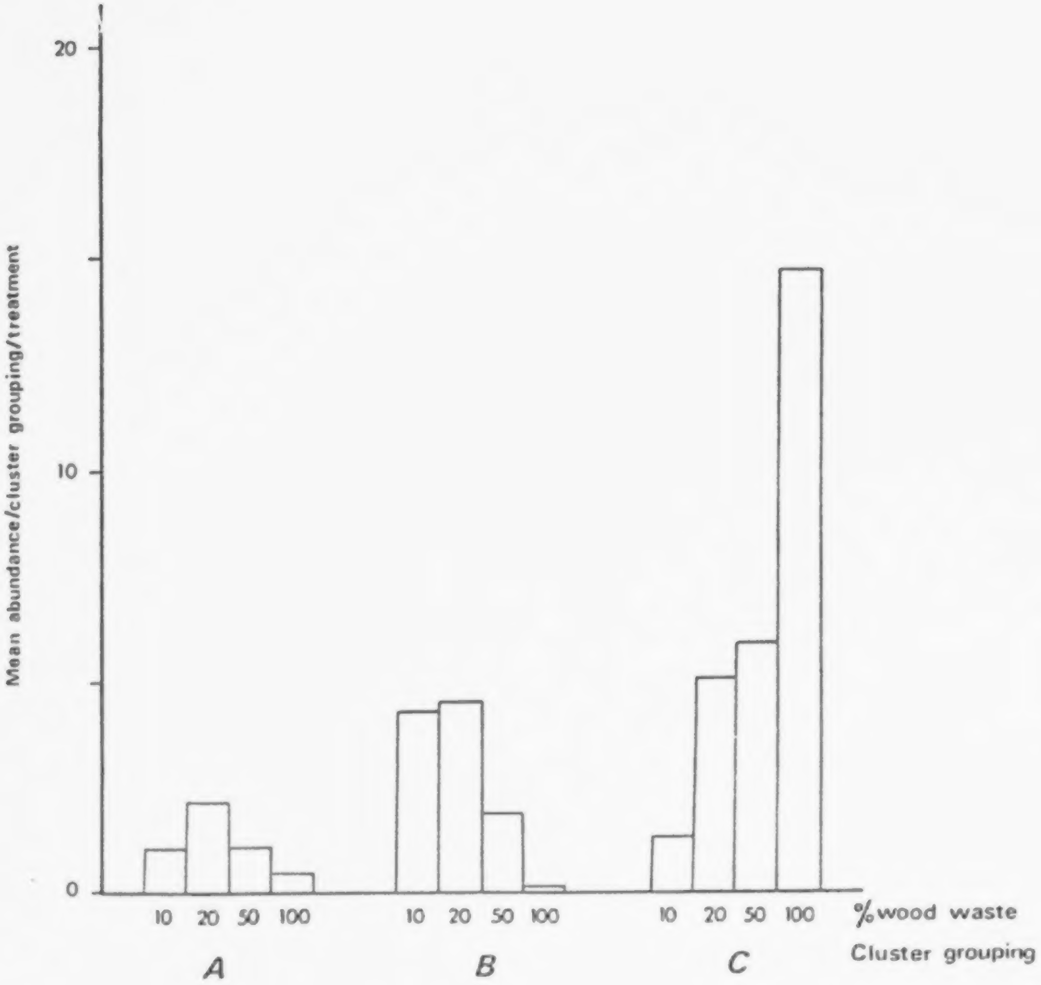

Figure 8. Mean number of individuals for each cluster analysis species grouping. Data derived from Fig. 6.

Table 1. Total number of species and mean number of individuals collected from each treatment for each major taxonomic group.

	Percent Wood Waste							
	0		20		50		100	
	Taxa.	Indiv.*	Taxa.	Indiv.*	Taxa.	Indiv.*	Taxa	Indiv.*
Nematoda	-	2.75	-	11.88	-	6.38	-	2.50
Oligochaeta	2	2.38	4	7.25	-	1.50	-	0
Polychaeta	24	22.25	41	31.38	2	21.50	11	59.00
Ostracoda	-	0.88	-	0.63	-	0.88	-	0.50
Amphipoda	2	0.63	3	1.75	-	0	2	1.50
Isopoda	-	0	-	0	-	0	1	0.25
Leptostraca	-	0	-	0	-	0	1	0.25
Hydrozoa	1	0.13	1	0.25	-	0	-	0
Bivalvia (excl. *Bankia*)	9	17.63	13	19.88	9	9.00	2	2.00
Bankia setacea	1	0.50	1	6.25	1	12.75	1	18.00
Gastropoda	-	0	-	0	1	0.25	-	0

* Based on means of 8 replicates for 0%, 20% and 50%; and, means of 4 replicates for 100%.

Sample cluster analyses (Q-type) performed on all three data sets clearly differentiated two distinct groups of samples (Fig. 4, 5 and 6). The same trends were shown with (M1) and without (M2, M3) rare species. This report focuses on M3, the second edit data matrix, containing the 17 most common/abundant taxa. Group 1 (Fig. 6) contained all of the 0% and all but one of the 20% samples but only three 50% samples and none of the 100% samples. Samples of each concentration within the two major groups were unevenly distributed, indicating generally inconsistent replication among each of the eight samples from a specific concentration.

Species abundance data indicated enhanced recruitment at 20% wood waste, compared to reduced recruitment at 100% (Fig. 6). Cluster analysis by species (or R-type) for the M3 data matrix differentiated three groups of species as shown in Figure 7. A higher abundance of particular species (Fig. 8, Group A) was noted at 20% compared to the other wood waste concentrations. Equally high numbers were noted at 0% and 50%, with very few individuals at 100%. No oligochaetes were found in the 100% treatment. Although little is known about the ecology of the tubificid oligochaetes found in these samples (Limnodriloides spp.), it was obvious from Table 2 and Appendix C that some wood waste enhanced their occurrence. This enhancement may be due to the additional nutrients and bacteria provided by the wood waste (Dr. R.O. Brinkhurst, Institute of Ocean Sciences, pers. comm.).

Similar trends were seen for all of the polychaetes associated with low amounts of wood fibres (Fig. 7, Group A). Ophelina acuminata, Glycera capitata, Tharyx spp. and Lanassa venusta venusta occurred in large numbers at 0% and 20% wood waste, but at much lower numbers in 50% and 100% treatments. The bivalves Lucina tenuisculpta, Psephidia lordi, Mysella spp. and Cyclocardia ventricosa were distinctly limited by 50% and 100% wood waste concentrations, with only Mysella compressa occurring in one sample of 100% treatment.

Group B species (Fig. 7) were clearly sensitive to high concentrations of wood waste (Fig. 8). The one polychaete and four bivalve molluscs comprising this group occurred in high abundance at 0% and 20% concentrations, decreased at 50%, and only the bivalve Mysella compressa was found at 100% wood waste. Abundances of these species in 0% and 20% concentrations were at least double those for other concentrations.

Group C species (Fig. 7) was comprised of nematodes, three polychaete taxa and the wood-burrowing shipworm Bankia setacea. There was a significant increase in all individuals of this group from 0% to 100% concentrations (Fig. 8). The large numbers of nematodes in the 20% concentration contributed to the high number for this concentration in Group C.

Data on particle size, total nitrogen and total organic carbon in the sediments are shown in Table 2. Substrate particle sizes were highly variable. The amount of sand increased from the 0% to the 50% samples with concomitant decreases in silt and clay. Total nitrogen decreased from the 0% to the 100% samples, while total organic carbon values were variable. Contrary to the faunal data matrices, a correlation matrix incorporating these variables indicated that they did not significantly contribute to the distribution of species among the four wood fibre concentrations. The faunal distribution was due, therefore, to physical or other parameters not measured (e.g. nutrients, bacteria).

Table 2. Substrate particle size, total nitrogen and total organic carbon in each concentration of wood waste.

Parameter	0%	20%	50%	100%
Particle Size				
Sand (.063-2 mm)	30.8	58.7	68.5	--
Silt (.004-.063 mm)	35.3	18.7	13.4	--
Clay (<.004 mm)	33.9	22.6	16.1	--
Total Nitrogen	0.14	0.06	0.04	0.02
Total Organic Carbon	4.21	7.58	6.70	46.20

All values expressed as percentages.

DISCUSSION

Most investigations of recolonization have described disturbance-related events (see, for example, Bonsdorff, 1980; McCall, 1977; Pearson and Rosenberg, 1978), although some studies have dealt specifically with benthic colonization using *in situ* sampling techniques (Arntz and Rumohr, 1982, Rumohr, 1982). The present study is discussed primarily with respect to the latter investigations.

The two dominant groups of recolonizing organisms collected during this study were polychaete worms and bivalve molluscs. Two other groups, nematodes and oligochaete worms, showed secondary importance in the initial or pioneering stage of benthic recruitment (see Table 1 and Fig. 6). In a three year study of benthic succession and seasonal variation in the Baltic Sea, Arntz and Rumohr (1982) found that polychaetes were always the most abundant organisms, followed by molluscs, regardless of exposure time. Crustaceans ranked third in abundance. In neither study were other taxonomic groups of major significance to the overall pattern of colonization or variation. In the present study, elimination of 48 (first edit; M2) or 64 (second edit; M3) occasional taxa from a total of 81 (original) taxa did not affect the patterns and trends for species richness, diversity, abundance, and cluster and ordination classification.

Pronounced variability was observed among samples for each concentration of wood waste tested. Four subsamples from a given concentration never clustered together, although they generally occurred within a single major cluster group. Several factors could have caused these differences among replicates. Because natural spatial dispersion of benthic invertebrates is usually contagious (Elliott, 1977), composition and abundance is often different among replicates. This effect can be compounded if small samples within a small area are taken. The short (i.e. 11 week) exposure time may have prohibited establishment of a stable community structure throughout the experimental containers, resulting in further patchiness. Differences in substrate characteristics (e.g. particle size, organic content, pH) among containers could also have encouraged the establishment of different faunal assemblages. As Rumohr (1980) and Arntz and Rumohr (1982) point out, those organisms which settle first have an advantage over those still in the plankton, while certain planktonic larvae will only settle after certain other species have already established themselves.

Based on the results of this study, the potential for benthic invertebrate colonization was enhanced if there was an approximate 1:3 ratio of wood waste to natural sediment. This combination of sediment and wood waste may increase available niches and provide more nutrients than sediments with no wood waste or too much wood waste. The upper percentage limit of beneficial mixtures of wood waste and sediment lies between 20% and 50%. Overall, the 20% samples showed high diversity, species richness and evenness, and low dominance. The 50% samples showed an opposite trend. It was concluded that wood waste additions in the range between 10% and 40% enhance benthic invertebrate colonization, provided that sediments/wood waste were well mixed (as in the experimental containers).

Only a few species (*Capitella capitata*, *Armandia brevis*, *Prionospio cirrifera*) were found in the 100% wood waste but their occurrence in large numbers indicated successful colonization. Numerous studies have shown *C. capitata*, to be dominant in polluted areas and a good biological indicator species for high levels of organic enrichment (see, for example, Pearson and Rosenberg, 1978; Reish, 1959 and 1980; Reish and Barnard, 1960; Rosenberg, 1973; Wade et al., 1972 and others). No *C. capitata* were collected in the 0% samples, very few in the 20% and 50% samples (mean value of one per sample), while the 100% samples had a mean value of 26 per sample. Ellis (1970) found *C. capitata* almost exclusively in a B.C. pulp mill fibre bed. *A. brevis* increased from a mean of two per sample at 0% to a mean of 21 per sample at 100%. A similar but less significant increase from 0% (mean of one worm) to 100% (mean of six worms) occurred with *P. cirrifera*. Both *A. brevis* and *P. cirrifera* have been associated with fauna occurring in organically polluted areas (Bagge, 1969; Chapman et al., 1982; Comiskey et al., 1984; Leppakowski, 1971). Reish (cited in Pearson and Rosenberg, 1978) found *P. cirrifera* present where *Capitella* spp. were dominant.

Increases in numbers of *Bankia setacea*, the wood-burrowing shipworm (bivalve), would be expected as the wood content increased. A mean number of 0.4 early settling larvae were found in the 0% samples, increasing to 9, 13 and 18 in 20%, 50% and 100%, respectively, with some small adults in the 100% samples.

Data from the present study and from infaunal core samples taken by Conlan (1977) showed similar species and sample cluster groups. Using both quantitative and qualitative clustering techniques, Conlan's (1977) data fell into distinct groupings for samples of control, intermediate and thick, wood-fibre mats. Consistent with our findings, she found large numbers of *Capitella capitata*, *Armandia brevis* and *Bankia setacea* present in areas of dense fibre mats, while *Mysella tumida* and *Psephidia lordi* were dominant in the controls.

Conlan (1977) found a positive correlation between species presence and trophic relationships in areas of different thicknesses of fibre mats, in a continuous progression from suspension feeders in control areas, to deposit feeders in moderately thick fibre areas, and herbivores in the areas with the most wood content. The species most closely associated with the present 100% samples (Fig. 7) were also deposit feeders or herbivores. However, the wide variety of feeding guilds (carnivore/scavenger, deposit feeder, suspension feeder) represented by those species associated with the 0% and 20% samples may indicate that it was not the type of feeding habit but rather the amount of wood wastes, which determined numerical presence. Habit showed no correlation with a particular cluster group, and habitat was, of course, the same for all samples. The above comparisons further indicated that the 20% wood wastes used in this study enhanced species abundances and diversity regardless of specific ecological and trophic relationships.

RECOMMENDATIONS

Present study

Wood wastes mixed in approximately a 1:3 ratio with sediments would enhance the potential for benthic invertebrate colonization and provided the concentration of seasoned wood in dredge spoils does not exceed 25% and is well mixed with the natural sediments, there should be no significant ecological damage.

Future studies

Several major questions have come to light during this investigation which warrant additional study, including: [1] what are the effects of green vs. aged wood waste, wood species and wood waste size distribution on benthic recolonization; [2] what are the effects of natural sediments overlain by different depths of wood wastes (unmixed) on benthic colonization; [3] what are the available organics, nutrient availability and time for breakdown in wood wastes mixed with sediments; and, [4] how does the in situ sampler compare with infaunal core samples from areas of wood deposition?

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STUDIES ON CHINOOK SALMON (ONCORHYNCHUS TSHAWYTSCHA) AND MUNICIPAL WASTE FROM THE IONA ISLAND SEWAGE TREATMENT PLANT, VANCOUVER

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Juvenile chinook salmon (*Oncorhynchus tshawytscha*) were exposed at the Iona Island sewage treatment plant to dilutions of effluent of 1, 5 and 15% for two months. Experiments were carried out under continuous flow conditions using diluent water drawn from the North Arm of the Fraser river. Effluent (unchlorinated and chlorinated) was pumped directly from sedimentation ponds at the sewage treatment plant.

A significant enhancement of growth at 5% effluent concentrations was accompanied by an apparent precocious sexual development in male chinook salmon. Increases in plasma sodium and reductions in muscle water levels suggest that exposure to municipal wastes can interfere with the ability of chinook salmon to adapt to sea water. Three widely differing responses to the combined effects of effluent exposure and common fish pathogens were also observed. These results emphasize the potential complexity of disease/effluent interactions and identify the need for a better understanding of the more subtle effects of chronic effluent exposure on fish health and subsequent survival.

A number of compounds toxic to aquatic life were identified in the unchlorinated sewage. Foremost among these were tetra- and pentachlorophenol and a series of PAH's in the 150 and 400 molecular weight range. Preliminary analysis of tissue samples from fish exposed to sewage showed uptake of the chlorinated phenols and a wide variety of PAH's. Nonylphenol and a series of unknown compounds that apparently contain chlorine have also been observed.

ÉTUDES SUR LE SAUMON CHINOOK (ONCORHYNCHUS TSHAWYTSCHA) ET SUR LES DÉCHÊTS MUNICIPAUX DE L'USINE DE TRAITMENT DE DECHÊTS DE L'ÎLE IONA, VANCOUVER

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Des saumons chinook juvêniles furent exposés pour deux mois à des dilutions d'effluent de 1, 5 et 15% à l'usine de traitement de l'île Iona. Les expériences furent conduites sous des conditions de flot continu, en utilisant de l'eau tirée du bras nord de la rivière Fraser. L'effluent (chloré et non chloré) était pompé directement des bassins de sédimentation situés à l'usine de traitement de déchets.

Chez les saumons chinook mâles, on nota une augmentation significative dans la croissance à des concentrations d'effluent de 5%, accompagnée d'un apparent développement sexuel précoce. Des augmentations de sodium dans le plasma et des réductions de la proportion d'eau dans les muscles suggèrent que l'exposition aux déchets municipaux peut nuire à la capacité d'adaptation à l'eau salée du saumon chinook. On observa aussi trois réponses très différentes aux effets combinés d'exposition à l'effluent et à des agents pathogènes communs chez les poissons. Ces résultats mettent en évidence la complexité potentielle des interactions entre maladie et effluent, et la nécessité d'une meilleure compréhension des effets plus subtiles de l'exposition chronique à des effluents sur la santé des poissons et sur leur survie subséquente.

Un certain nombre de composés toxiques à la vie aquatique furent identifiés dans les déchets non chlorés. Le têtra- et le pentachlorophénol, ainsi qu'une série de HPA de poids moléculaire variant de 150 à 400, étaient prédominants parmi ces composés. Des analyses préliminaires d'échantillons de tissus de poissons exposés aux déchets démontrèrent une absorption de phénols chlorés et d'une grande variété de HPA. On observa aussi du nonylphénol et une série de composés inconnus qui contiennent apparemment du chloré.

The discharge of sewage treatment plant effluents into intertidal waters can lead to progressive habitat degradation. This paper describes field observations of the ecological impact of effluent discharged from the Iona Island Sewage Treatment Plant on fish and fish habitat in the Fraser River estuary. The rationale and an outline of an interdisciplinary laboratory study of the sublethal effects of effluent on juvenile chinook salmon is also provided. Preliminary results of efforts to chemically characterize effluent components, to investigate uptake of selected organic contaminants by salmon and to determine physiological effects on growth and salinity tolerance are discussed.

The Iona Island Sewage Treatment Plant, located by the North Arm of the Fraser River, is the largest of four primary treatment plants operated by the Greater Vancouver Sewerage and Drainage District. Built between 1961 and 1963, and expanded three times since then, the plant services 640,000 people and discharges directly into the upper intertidal zone of Sturgeon Bank, an important fish rearing habitat in the Fraser River estuary.

At low water, a 7 km-long dredged channel conveys effluent seaward and also limits its lateral spread onto the exposed banks, while a rock jetty parallel to the dredged channel and extending 4 km seaward into the Strait of Georgia restricts the northward movement of the effluent towards bathing beaches. Dilution at high water is further limited by oceanographic features of a shallow, highly stratified estuary.

Discharge rates vary seasonally from 4 m^3/s (53,000 Imp. gpm) average dry weather flow, to 17.7 m^3/s (234,000 gpm) during wet weather periods. Flows in excess of the current primary treatment capacity of 17.7 m^3/s (195,000 gpm) are discharged untreated into receiving waters. All effluent is chlorinated from May until September to control bacterial contamination of the receiving waters.

Because of a relatively small industrial input (6-8%), the acute toxicity of Iona effluent was found to be relatively low; 96 h LC_{50} of 40-45% v/v for sockeye salmon fingerlings (Martens and Servizi, 1976). However, the continous input of effluent at a flow rate in the range of that of a small river has led to a severely degraded receiving water environment. Studies conducted over a period of 10 years have included water and sediment chemistry, bacteriology and heavy metal contamination of sediments and of organisms utilizing the area.

During the course of these studies, it became apparent that in the summer months, there occurred frequent fish kills over an area of several square kilometers. In 1980, we conducted cage studies with juvenile chinook salmon and confirmed acutely toxic conditions. Concurrent measurements of dissolved oxygen indicated a serious oxygen depression over a widespread area of receiving waters. In spite of this inhospitable environment, the utilization of this area by fish, crabs, mysids and a variety of other organisms was continuing and this led to regular outright kills and frequently to "ecological death" from predation by gulls and herons on fish exhibiting abnormal behaviour. These effects and their potential significance to fishery resources were reported by Birtwell et al. (1983). The importance of Sturgeon Bank as fish habitat was recorded by Greer et al. (1981), who captured 38 species of fish including all 5 species of Pacific salmon; chinook salmon being the most common salmon species.

It is known that certain stocks of Fraser River chinook salmon spend up to two months rearing in the surface waters of the intertidal zone which are currently being used for effluent dilution. Since Fraser River chinook stocks continue to decline from historical levels, in 1982 we initiated a study of sublethal effects of Iona effluent on juvenile chinook salmon to determine whether effluent exposure might reduce their chances of survival during seawater acclimation and estuarine rearing.

The study included an analytical chemistry component to screen effluent and fish tissue for selected contaminants by GC-MS. Figure 1 indicates the scope of the effort to study responses at increasing levels of biological organization (Sprague, 1971). Figure 2 provides an outline of the various components and indicates the interdisciplinary nature of the experimental approach.

Initial experiments conducted in the summer of 1982 were discontinued after one month due to interruption of diluent water flow for several hours and the subsequent discovery of an infection of all fish with the myxosporidian parasite *Ceratomyxa shasta*. Ceratomyxosis is a terminal disease in Pacific salmon and we confirmed that the infective stage was present in diluent water taken from the Fraser River. The combination of stress from *C. shasta*, a doubling of effluent concentration in exposure troughs for approximately two hours and a slight reduction in dissolved oxygen levels (to 6.5 mg/L) subsequently led to dose-dependent mortalities over the next 48 hours. This response indicates that infected fish were less resistant to the stress of effluent exposure.

The results of seawater challenge tests conducted, albeit with chronically *C. shasta* infected fish, suggested that effluent exposure reduced the ability of juvenile chinook salmon to regulate plasma sodium (elevated) and muscle water (decreased) indicating hydromineral imbalance.

The study was repeated in 1983 using diluent water sterilized with ultraviolet light. A disease screening protocol was included and showed that *C. shasta* was absent in fish living in effluent diluted with UV-sterilized Fraser River water. A concurrent experiment conducted with UV-bypass water once again demonstrated 100% infection with *C. shasta* in a stock of chinook salmon indigenous to the Fraser River system.

In 1983, we observed widely differing responses to combined effects of effluent exposure and a myxobacterial/furunculosis infection apparently already present in latent form in this hatchery-released chinook salmon stock. Figure 3 indicates identical mortality rates at 0, 1 and 5% effluent; increased mortalities at 15% effluent suggest a possible threshold in resistance during the myxobacterial disease.

In marked context, during the furunculosis outbreak, mortality rates, except for 15% again, appeared to be *inversely* related to effluent exposure. We can only speculate that perhaps previous effluent exposure stimulated the immune system enabling the fish to deal more effectively with the furunculosis infection. An alternate hypothesis could be the presence of antibiotic agents in the effluent assisting in the control of furunculosis in salmon, a disease which normally is treated with oxytetracycline administered in the diet.

The chemical characterization of Iona effluent was conducted on liquid/liquid extracts of composite samples pooled every 5 days. Continuous effluent

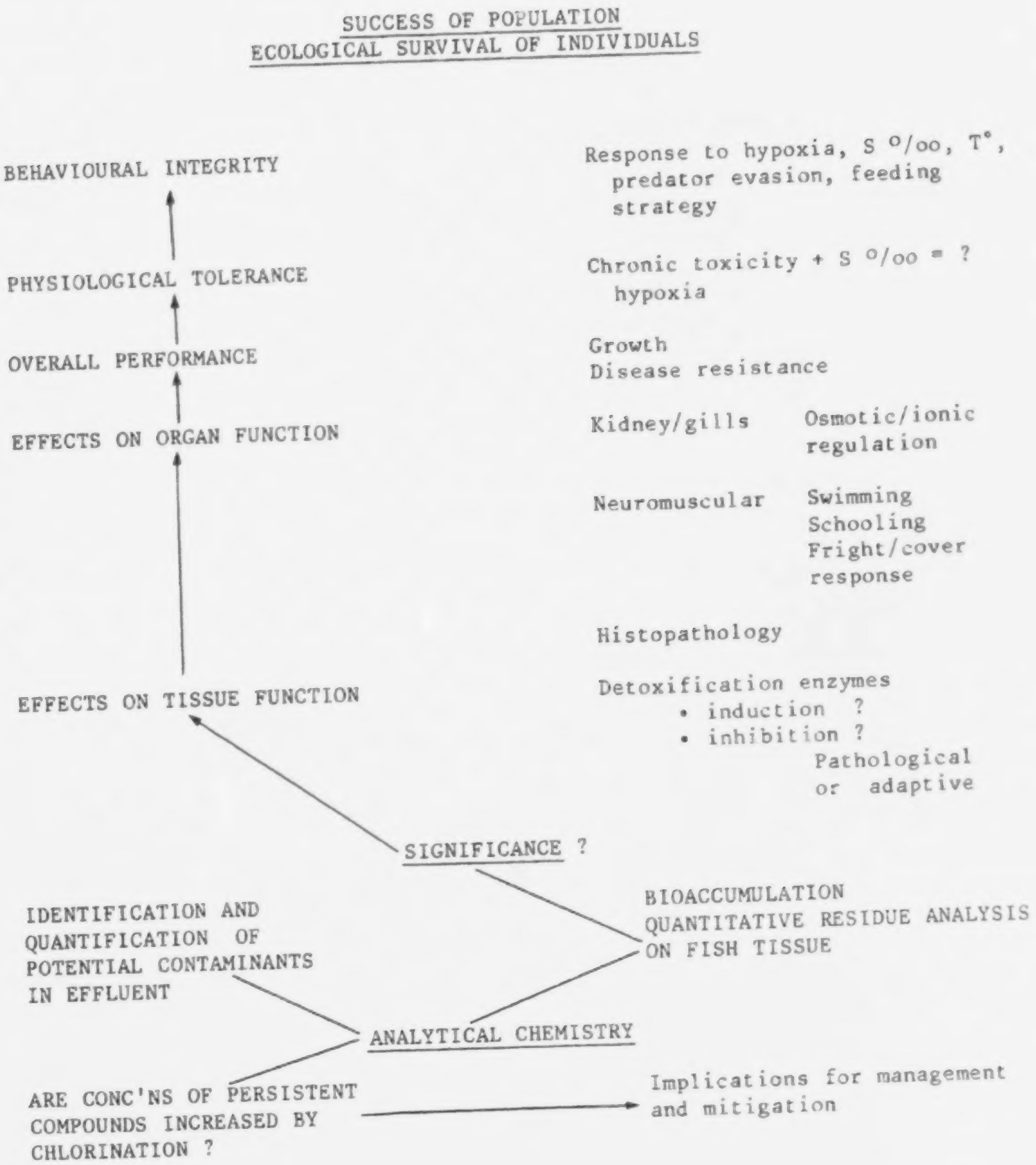
SUCCESS OF POPULATION
ECOLOGICAL SURVIVAL OF INDIVIDUALS
BEHAVIOURAL INTEGRITY
Response to hypoxia, S °/oo, T°, predator evasion, feeding strategy
PHYSIOLOGICAL TOLERANCE
Chronic toxicity + S °/oo = ? hypoxia
OVERALL PERFORMANCE
Growth
Disease resistance
EFFECTS ON ORGAN FUNCTION
Kidney/gills
Osmotic/ionic regulation
Neuromuscular
Swimming
Schooling
Fright/cover response
Histopathology
EFFECTS ON TISSUE FUNCTION
Detoxification enzymes
• induction ?
• inhibition ?
Pathological or adaptive
SIGNIFICANCE ?
BIOACCUMULATION
QUANTITATIVE RESIDUE ANALYSIS ON FISH TISSUE
IDENTIFICATION AND QUANTIFICATION OF POTENTIAL CONTAMINANTS IN EFFLUENT
ANALYTICAL CHEMISTRY
ARE CONC'NS OF PERSISTENT COMPOUNDS INCREASED BY CHLORINATION ?
Implications for management and mitigation

Figure 1. Components of a study of sublethal effects, at increasing levels of biological organization, of Iona Sewage Treatment Plant effluents on juvenile chinook salmon.

IONA 1983

June 4 - August 15

		C	UC
BEHAVIOUR/ECOLOGY PHYSIOLOGY BIOCHEMISTRY HISTOLOGY ENDOCRINOLOGY			
	Survival	+	+
	Growth	+	+
	Behaviour	+	+
	Sea Water Tolerance	+	+
	Plasma Cortisol		+
	Interrenal Histology		+
	Enzyme Activity UDP-GT	+	+
ANALYTICAL CHEMISTRY	Weekly -		
	Tissue Sampling for Organic		+
	Contaminants and Metals		+
	Effluent Analysis - Continuous Weekly Composite For Profile of Trace Organic Contaminants	+	+
	Weekly Sampling for Metals		+
	Fraser River Water 2 × 400 L		
HEALTH/PATHOLOGY	Organ Histopathology		+
	Ceratomyxa shasta		+
	Disease Screening		+
	Disease Challenge		+
BEHAVIOUR/FEEDING	Utilization of Degraded Habitat		

Figure 2. Outline of experimental design of an interdisciplinary study of sublethal effects of Iona Sewage Treatment Plant effluents on juvenile chinook salmon. Exposure was to a continuous flow of 0, 1, 5 and 15% effluent diluted with North Arm Fraser River water. **C** indicates chlorinated; **UC** unchlorinated effluent.

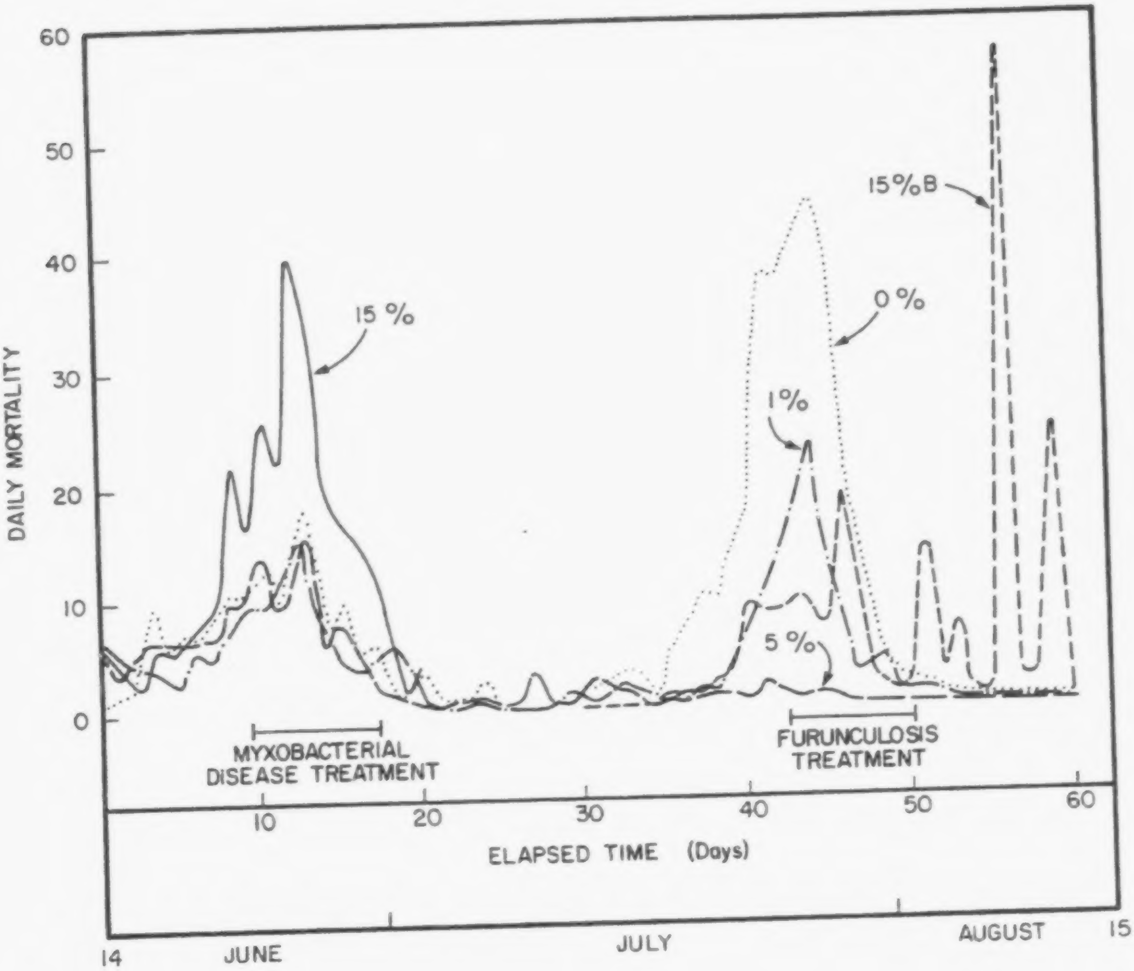

Figure 3. The influence of disease on mortality rates of juvenile chinook salmon during exposure to dilutions of municipal waste. 1983.

collection provided 25 L per day. Selected contaminants were quantified in effluent and in fish sampled every 10 days during the exposure period. Table 1 provides a partial list of organic contaminants that were found in effluent and are known to exhibit environmentally persistent or toxic properties at low concentrations.

Figure 4 illustrates the concentrations of two chlorinated organic contaminants, tetra- (TCP) and pentachlorophenol (PCP) in unchlorinated primary-treated effluent during the course of the experiment. A spill in the week of July 20-25th was reflected in elevated PCP residues in fish (120 µg/kg and 260 µg/kg wet weight) exposed to 5% and 15% effluent respectively. Tissue levels of PCP

Table 1. A partial list of contaminants identified in Iona effluent which can pose a threat to fish health.

Tetrachlorophenol
Pentachlorophenol
Nonylphenols
Cresols
Dimethylphenol
Ortho-phenyl phenol
Nonylphenolethoxylates
Phthalate esters

Dehydroabietic acid
Naphthalene
Phenanthrene
Methylnaphthalenes (N = 1,2,3)
Methylphenanthrenes (N = 1,2,3)
Methyldibenzothiophenes (N = 1,2)
PCBs

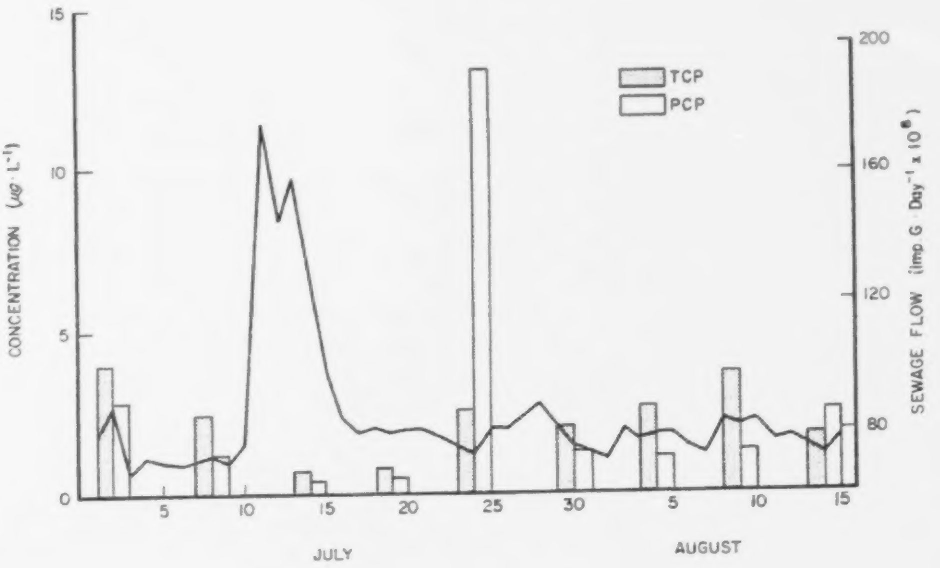

Figure 4. Tetra- and pentachlorophenol levels in unchlorinated sewage taken from primary sedimentation pond at Iona Sewage Treatment Plant 1983. Each sample comprised a 5-day composite collected continuously. Identification and quantification by GC-MS.

remained elevated relative to controls for the next 20 days. A series of 6 unknown organic compounds, believed to be aromatic non-halogenated contaminants, were also found to be accumulating in fish tissue in a dose-dependent fashion. A similar set has been detected in Wisconsin carp (Dr. P. Peterman, personal communication) but remain unidentified.

The growth of juvenile chinook salmon was markedly increased by effluent exposure (Figure 5) and was concentration-dependent.

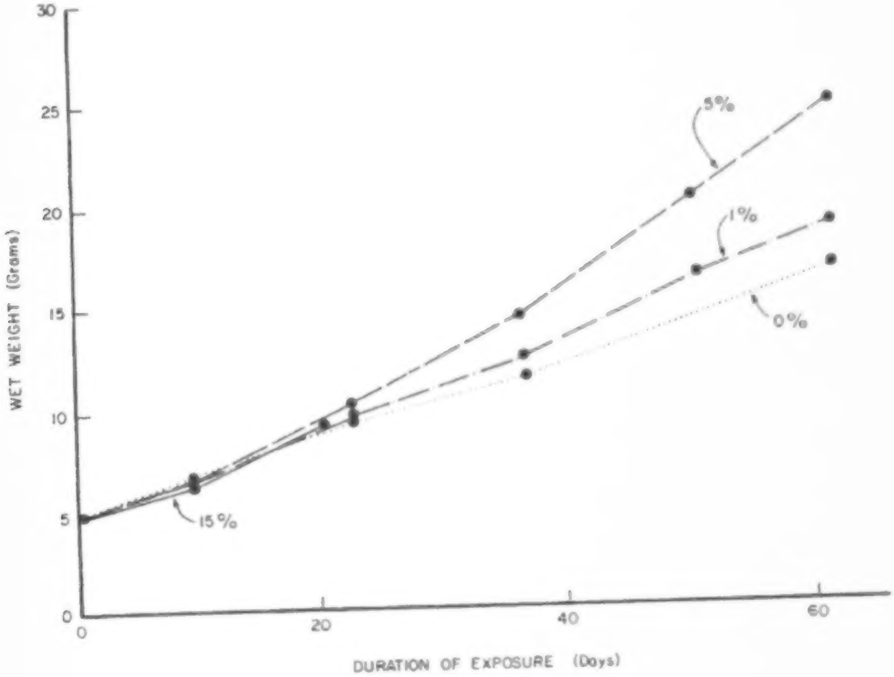

Figure 5. Growth of juvenile chinook salmon in dilutions of Iona municipal waste and Fraser River water. The 15% group was terminated due to technical problems. Each point represents mean weight of 125-130 fish.

One hypothesis to explain growth enhancement and an observed apparent precocious development in male salmon exposed to 5% effluent might be the presence of androgenic compunds at low concentrations. Androgenic effects on mosquito fish have been reported in waters receiving pulp mill waste effluents (Howell et al., 1980). One such candidate compound, β-sitosterol, is known to be present in sewage wastes (Hatcher et al., 1977) and has been implicated by Howell (personal communication) in the androgenic activity of some pulp mill effluents. Other compunds possessing androgenic activity and likely to be found in sewage are progestins, components of oral contraceptives which are derivatives of 19-α-nortestosterone and are excreted in human waste.

Weekly tests of the seawater tolerance of fish exposed to Iona effluent showed a progressive reduction in survival at 15% and a drop in muscle water levels in 5%-exposed fish. Plasma ion analyses presently underway should help determine the extent of this hydromineral imbalance.

The activity of a liver enzyme responsible for detoxification of a variety of xenobiotics, UDP-glucuronosyl transferase (UDP-GT) was induced by effluent exposure (Figure 6) and indicates an increase in detoxification activity of chinook salmon liver.

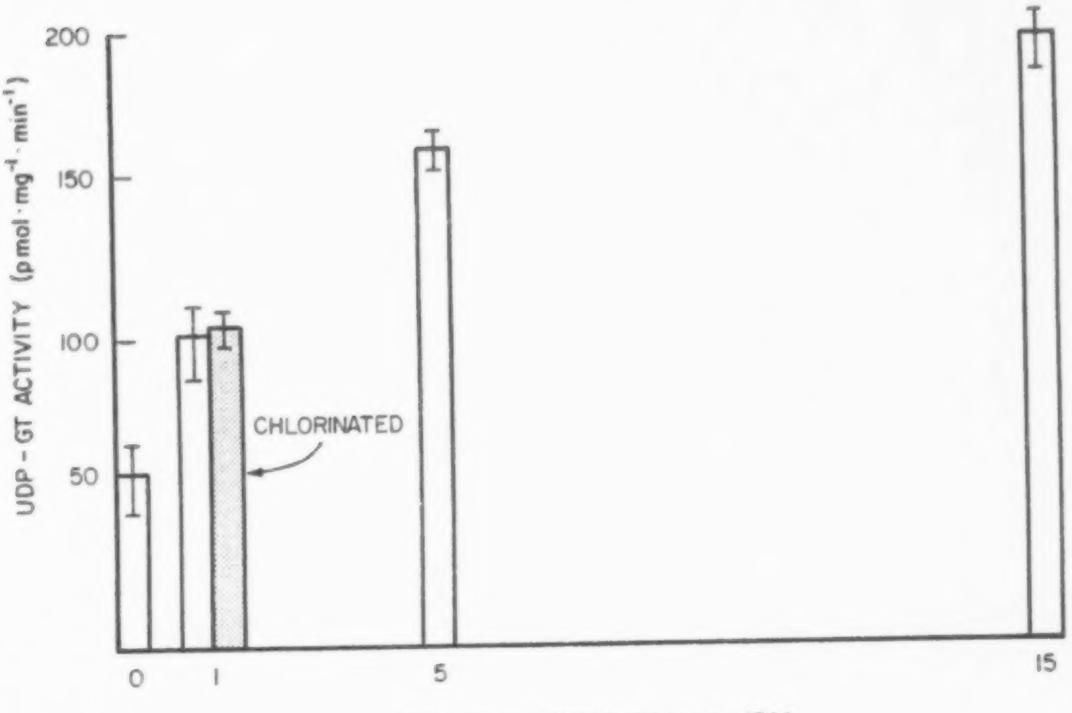

Figure 6. UDP-glucuronosyl transferase activity in livers of juvenile chinook salmon exposed to dilutions of Iona municipal waste for 62 days. Exposure time for 15% was 29 days. Each bar represents mean ± SE of 5 pooled homogenates of 5 livers each.

We hope to have the results of the rest of these experiments completed within the next six months and should be better able to interpret the preliminary results presented today.

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THE INTERACTION OF SELENATE AND SELENITE WITH SELECTED FRESHWATER SEDIMENTS

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Submicrogram levels of selenite and selenate were added to suspensions of two lake sediments from the Qu'Appelle river basin in southeastern Saskatchewan. The reactions were conducted in a shaker bath at 4, 25 and 60°C. The sediment from Buffalo Pound Lake was found to rapidly oxidize selenite to selenate; the oxidation followed the Arrhenius type reaction and obeyed multiple first order kinetics. The sediment from Katepwa Lake also oxidized selenite, but at a much slower rate. Selenite was strongly adsorbed by the sediments and the selenium remaining in solution was minimum at 4°C. The data indicate that the oxidation of selenite by these sediments is an abiotic process.

Selenate was rapidly reduced to selenite by the Katepwa Lake sediment at 25°C. The reduction was considerably slowed down at 4°C and did not occur at 60°C. Buffalo Pound Lake sediment did not reduce selenate. Selenate is not adsorbed by either sediment. The reduction of selenate by Katepwa Lake sediment is likely owing to biotic activity or associated biochemical processes.

Because these lakes have similar basic mineralogy, climatic conditions, and anthropogenic inputs, their differences in the redox behavior of selenium compounds may be explained by the chemical and biological milieu created by differing physical characteristics of the lakes.

L'INTÉRACTION ENTRE LE SÉLÉNIATE, LE SÉLÉNITE ET CERTAINS SÉDIMENTS D'EAU DOUCE

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Des quantités de moins d'un microgramme de sélénite et de séléniate furent ajoutées à des suspensions de deux sédiments lacustres du bassin de la rivière Qu'Appelle, dans le sud-est de la Saskatchewan. Les réactions furent menées dans un bassin secoueur à 4, 25 et 60°C. Le sédiment du lac Buffalo Pound oxidait rapidement le sélénite en séléniate; l'oxidation était une réaction de type Arrhénius et obéissait aux lois de la cinétique de premier ordre multiple. Le sédiment du lac Katepwa oxidait aussi le sélénite, mais à une vitesse moindre. Le sélénite était fortement absorbé par les sédiments et le sélénium restant dans la solution était minimal à 4°C. Les données indiquent que l'oxydation de sélénite par ces sédiments est un processus abiotique.

Le séléniate était rapidement réduit en sélénite par le sédiment du lac Katepwa à 25°C. La réduction était considérablement ralentie à 4°C et n'avait pas lieu à 60°C. Le sédiment du lac Buffalo Pound ne réduisait pas le séléniate. Le séléniate n'est absorbé par aucun des sédiments. La réduction du séléniate par le sédiment du lac Katepwa est probablement due à l'activité biotique ou associée à des processus biochimiques.

Parce que ces lacs ont une minéralogie de base, des conditions climatiques et des sources d'apports similaires, leurs différences en oxydoréduction pour les composés de sélénium peuvent être expliquées par les milieux chimiques et biologiques créés par différentes caractéristiques physiques de ces lacs.

INTRODUCTION

The transformations of Se in natural systems has been a subject of study for some years. The reason for this interest owes to the importance of Se as an essential nutrient for animals and human beings as well as its reputation as a toxin similar in effect to arsenic (Demayo et al., 1979; Underwood, 1977; Fleming, 1980). Selenium is involved with antagonistic and synergistic reactions affecting the toxicity of metals such as Hg, As and Cd (Underwood, 1977; World Health Organization, 1978). It has also been implicated in the prevention of several forms of cancer, multiple sclerosis and heart disease (Oldfield et al., 1974; Pechter, 1981; Jackson and Lim, 1982).

Much of the research concerning Se in natural systems has been concentrated on adsorption by soil particles and plant uptake (Kubota et al., 1967; Cheng et al., 1980; Fleming, 1980; Watkinson, 1981). In addition, the interactions of Se with microbial species have been studied (Chau et al., 1976; Sarathchandra and Watkinson, 1981; Lindblow-Kull et al., 1981). Relatively little is known about the reactions of Se in freshwater sediments, however (Demayo et al., 1979).

Huang et al. (1982) reported that sediments from Katepwa Lake in the Qu'Appelle River basin in Saskatchewan was capable of reducing selenate to selenite, while the sediment from Buffalo Pound Lake in the basin performed the opposite reaction. They proposed that these transformations of Se would affect its solubility, toxicity and subsequent contamination of the food chain.

The objective of this study was to investigate the kinetics of the oxidation of selenite and the reduction of selenate at varying temperatures by the sediments of the Katepwa and Buffalo Pound Lakes in Saskatchewan. This information would facilitate the fundamental understanding of the mechanisms involved in the redox behaviours of Se in the systems. Furthermore, it is essential to understand seasonal variations of the redox reactions of Se and its subsequent mobility in freshwater ecosystems.

MATERIALS AND METHODS

Sediments

Sediments from two freshwater lakes (Buffalo Pound and Katepwa) located in the upper Qu'Appelle River Basin in southeastern Saskatchewan, Canada were selected for this study. The nature of the lakes and sediments are described elsewhere (Oscarson et al., 1981). Bottom lake sediment samples were collected with an Ekman dredge. Several sediment samples from each lake were combined and thoroughly mixed to make a composite sample; the composite sample of each lake sediment was used for all experiments. The sediment samples were stored in sealed plastic bottles at 4°C.

Reagents

Reagent grade Na_2SeO_3 and Na_2SeO_4 were standardized gravimetrically (Erdey, 1965). The procedure given by Wilkie and Young (1970) was followed for the preparation of the 2,3-diaminonaphthalene (DAN) complexing reagent.

Redox reactions of selenite and selenate

Three grams (oven-dried weight basis) of sediment samples were suspended in 300 ml of a solution that contained 100 ng/ml of Se as Na_2SeO_3 or Na_2SeO_4 in 500 ml Erlenmeyer flasks. Each experiment was run in duplicate.

For all experiments, the flasks containing the suspensions were stoppered and placed in a water bath on an oscillating shaker. The temperature of the bath was adjusted to 4, 25, or 60°C. At various time periods up to a maximum of 5 weeks, the suspensions were thoroughly mixed and a 25 ml aliquot of each suspension was withdrawn. This sample was centrifuged at 1,400 g for 20 min and the supernatant was filtered (0.45 µm pore size). Five milliliters of the filtrate was analyzed fluorometrically in duplicate for selenite and 5 ml in duplicate for selenite plus selenate as described by Huang et al. (1982) with minor modifications as described below.

The piazselenol complex was extracted with n-hexane from aqueous solution in 125 ml flat bottomed boiling flasks, replacing the separatory funnels used previously. These flasks were shaken on a wrist action shaker and the hexane extracts were decanted into fluorescence cuvettes. The boiling flasks and cuvettes were capped with cork stoppers.

To determine selenite plus selenate, the method of reduction proposed by Rankin (1973) was employed. This involved preoxidation of the sample with 30% H_2O_2 to remove organics, followed by heating and reduction to selenite by addition of concentrated HCl. The pH of the solution was adjusted to 1.8±0.2 with concentrated NH_4OH.

These changes were made to increase the speed of analysis. The detection limit using this procedure is about 1 ng Se/ml.

At the end of various reaction periods, the Eh (measured with a Pt electrode vs a AgCl/Ag reference electrode in 4M KCl) and pH of the suspensions were determined.

RESULTS AND DISCUSSION

The Katepwa Lake sediment was capable of reducing Se(VI) to Se(IV) at 4 and 25°C; the depletion of Se(VI) by the sediment was only observed at the initial stage of 60°C (Fig. 1). The Buffalo Pound Lake sediment lost its reducing ability (Fig. 2) since the original experiment was carried out (Huang et al., 1982). This is attributable to oxidation of the sediment during storage at 4°C. It was unable to reduce Se(VI) at any of the temperatures studied (Fig. 2).

The rate of the reduction reaction of Se(VI) by the Katepwa Lake sediment was very drastically decreased at 4°C and not observed at 60°C (Fig. 1, Table 1), indicating that the reduction reaction is mediated by biota, related biochemical processes and/or organic matter. This also reinforces a suggestion made earlier (Huang et al., 1982) that Se(VI) may serve as an electron acceptor in the oxidation of organic matter. The microbes, related biochemical processes and/or organic matter in the Katepwa Lake sediment appeared to differ in nature from the Buffalo Pound Lake sediment, since the former was able to mediate the

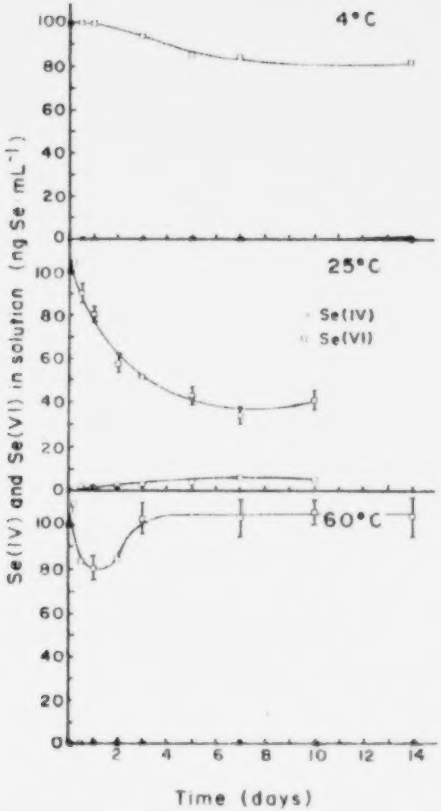

Figure 1. Effect of temperature on the reduction of selenate to selenite in Katepwa Lake sediment. The experiment at 4°C was extended to 35 d; the depletion of Se(VI) and the formation of Se(IV) continued slowly. If no deviation is indicated, the standard deviation is within the size of the symbol.

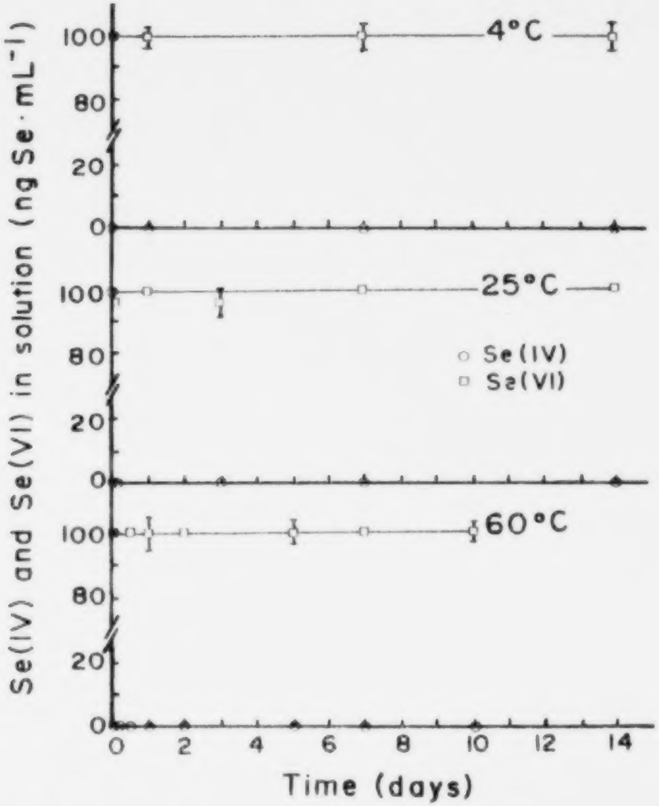

Figure 2. Effect of temperature on the reduction of selenate to selenite in Buffalo Pound Lake sediment. The experiment at 4°C was extended to 35 d; there was no observable change in the reaction. If no deviation is indicated, the standard deviation is within the size of the symbol.

Table 1. Rate constants and heat of activation of the oxidation of selenite and reduction of selenate by Buffalo Pound and Katepwa Lake sediments.

		Temperature (°C)			
		4	25	60	Heat of activation (kJ/mol)*
Transformation of Se species	Reaction phase	Rate constant $(d^{-1}) \times 10^{3}$*			
Buffalo Pound Lake sediment					
Se(IV) to Se(VI)	Fast	7.1 ± 3.0	70.0 ± 3.6	506 ± 19	57.8 ± 4.6
	Slow	3.3 ± 1.0	13.9 ± 0.7	42.8 ± 2.3	34.8 ± 4.6
Se(VI) to Se(IV)	Fast	0.00	0.00	0.00	---
	Slow	0.00	0.00	0.00	---
Katepwa Lake sediment					
Se(IV) to Se(VI)	Fast	0.95 ± 0.08	0.37± 0.03	---**	---**
	Slow	---	---	---	---
Se(VI) to Se(IV)	Fast	35.5 ± 1.1	283.0 ± 14.0	0.00	67.9 ± 3.1
	Slow	5.4 ± 0.4	104.0 ± 7.3	0.00	96.8 ± 7.4

*The oxidation of Se(IV) and the reduction of Se(VI) were calculated on the basis of the amounts of Se(IV) and Se(VI) remaining in the systems (solution and adsorbed phase).

**Competing reactions interfere with calculations of rate constants.

Table 2. pH and Eh values of the freshwater sediments studied.

	Buffalo Pound Lake Sediment		Katepwa Lake Sediment	
Reaction period	pH	Eh (mV)	pH	Eh (mV)
Beginning of reaction period	8.4	420	7.8	390
End of reaction period	8.0	420	7.7	430

reduction of Se(VI) while the latter could not.

It should be noted that both sediments were in a similar state of oxidation (Table 2). This indicated that Eh/pH status was not the primary cause of reduction in these systems.

The Katepwa Lake sediment after storage at 4°C was capable of oxidizing small amounts of SE(IV) to Se(VI), although the rate of the reaction did not vary substantially from 4°C to 25°C (Fig. 3, Table 1). At 60°C, the reaction appeared to become more complex. Large amounts of Se(VI) were initially formed and then disappeared. Competing reactions apparently occurred, and thus affected the equilibrium of Se(IV) and Se(VI) in the system.

Buffalo Pound Lake sediment displayed a striking ability to oxidize Se(IV) to Se(VI) (Huang et al., 1982). This trend was verified in the present study (Fig. 4). Furthermore, the rate of the oxidation of Se(IV) increased with increasing temperature from 4°C to 60°C (Table 1), indicating that substantial seasonal variations of the speciation of Se in water would occur. The heat of activation for the reaction was 57.6 kJ/mol for the fast reaction and 34.8 kJ/mol for the slow reaction (Table 1). The oxidation of Se(IV) by the Buffalo Pound Lake sediment followed the Arrhenius type reaction, indicating that the oxidation of Se(IV) by the Buffalo Pound Lake sediment is an abiotic process. Manganese dioxide did not catalyze the oxidation of Se(IV) over 7 wks even though the reaction is thermodynamically favourable (Huang et al., 1982). The reaction occurs far too rapidly for MnO_2 to be the primary catalytic agent. The components responsible for the observed oxidation of Se(IV) by the sediment remain to be uncovered.

The kinetics of the oxidation of Se(IV) by the Buffalo Pound Lake sediment as well as the reduction of Se(VI) by the Katepwa Lake sediment have two phases. The fast phase occurred over a short period of time, from 2 to 5 days; the length of the period decreased with increasing temperature. The slow phase continued to the end of the reaction period.

The Katepwa Lake sediment may both oxidize and reduce Se compounds. There must, therefore, be an equilibrium that is reached as each reaction proceeds. Theoretically, as the sediment becomes more oxidized, the overall reaction will favour the formation of Se(VI).

Neither sediment was capable of adsorbing significant amounts of Se(VI). On the other hand, Se(IV) had a strong affinity toward both sediments. The Katepwa Lake sediment had a higher sorption capacity for Se(IV) (Table 3).

Temperature at 4 and 25°C did not affect the sorption reaction in the Katepwa Lake sediment (Table 3). The oxidation of Se(IV) by this sediment was also not very temperature sensitive in the same temperature range (Fig. 3).

The sorption of Se(IV) by the Buffalo Pound Lake sediment, like its ability to oxidize Se(IV) to Se(VI), was also highly temperature sensitive. The adsorption decreased from 4° to 60°C by 3.7 times (Table 3). Even if the temperature decreased from 4°C to 25°C, the adsorption of Se(IV), decreased 2.8 times, indicating that the mobility of Se in the freshwater system would vary with seasonal variations of temperature. The rate of oxidation of Se(IV) to

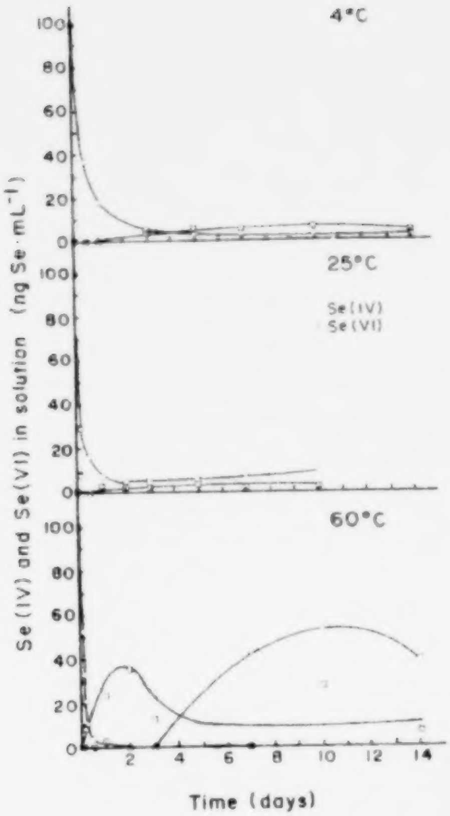

Figure 3. Effect of temperature on the oxidation of selenite to selenate in Katepwa Lake sediment. The experiment at 4°C was extended to 35 d; the depletion of Se(IV) and the formation of Se(VI) continued slowly. If no deviation is indicated the standard deviation is within the size of the symbol.

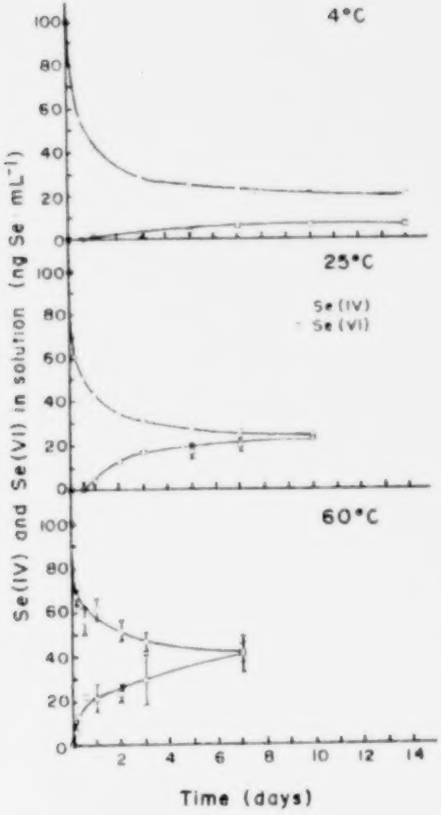

Figure 4. Effect of temperature on the oxidation of selenite to selenate in Buffalo Pound Lake sediment. The experiment at 4°C was extended to 35 d; the depletion of Se(IV) and the formation of Se(VI) continued slowly. If no deviation is indicated, the standard deviation is within the size of the symbol.

Table 3. Adsorption of Se(IV) by Buffalo Pound and Katepwa Lake sediments. Selenium in system after 7 days.

Reaction Temperature	Final pH	Final Eh (mV)	ng of Se(IV)/ml* of solution	ng of Se(VI)/ml* of solution	µg of Se adsorbed/g of sediment
Buffalo Pound Lake sediment					
4	8.0	420	23 ± 1.5	6 ± 3.6	7.1 ± 0.2
25	8.0	400	26 ± 1.8	20 ± 4.0	5.4 ± 0.4
60	8.1	400	41 ± 4.2	40 ± 11.4	1.9 ± 0.7
Katepwa Lake sediment					
4	7.7	430	3 ± 0.8	5 ± 1.5	9.2 ± 0.1
25	7.7	430	8 ± 1.2	1 ± 1.2	9.1 ± 0.1
60	7.7	420	43 ± 1.0	0 ± 0.0	5.7 ± 0.1

*the initital Se concentration in solution was 100 ng Se(IV)/mL and 0 ng Se(VI)/ml.

Se(VI) and the subsequent mobility of Se in freshwater systems would thus be higher in summer than winter.

These two sediments are similar in basic mineralogy (Oscarson et al., 1981). These two lakes are located approximately 100 km apart in the Qu'Appelle River basin and have similar natural and anthropogenic inputs. The only significant difference between them is their depth of water, and subsequent limnological environments, and the associated sediment characteristics. Buffalo Pound Lake is shallow (mean depth of 3 m) and its sediments are fairly well oxidized. Katepwa Lake sediments tend to be reduced as the deep lake becomes stratified (mean depth of 14.4 m). This difference appeared to produce a distinctly different chemical and biological milieu and, thus apparently affected the chemical and biological processes pertaining to the redox reactions and sorption of Se in the freshwater systems.

SUMMARY

The rates of the oxidation of Se(IV) and the reduction of Se(VI) and the sorption of these Se species at 4, 25, and 60°C in two freshwater sediments (Katepwa and Buffalo Pound Lakes) in the Qu'Appelle River basin in Saskatchewan were studied. The Katepwa Lake sediment reduced Se(VI) to Se(IV) at 25°C, but the reaction was drastically decreased at 4°C and was not observed at 60°C. This reduction reaction is attributed to the mediation of biota, related biochemical processes, and/or organic components of the sediments. Buffalo

Pound Lake sediment was unable to reduce Se(VI) at any temperature. Both Katepwa and especially Buffalo Pound Lake sediments were capable of oxidizing Se(IV) to Se(VI). The extent of oxidation caused by Katepwa Lake was relatively limited, and independent of temperature; competing reactions appeared to have occurred to affect the equilibrium of selenite and selenate in the system. The oxidation of Se(IV) to Se(VI) by the Buffalo Pound Lake sediment followed an Arrhenius type reaction, indicating that the oxidation process is abiotic in nature.

ACKNOWLEGEMENTS

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SOME EFFECTS OF SUSPENDED FRASER RIVER SEDIMENTS ON SOCKEYE SALMON

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Juvenile sockeye salmon were exposed to fine, medium and coarse sediments collected from sand bars in the Fraser River. Measurements of suspended solids were made during bioassays to document the exposure levels. Coarse sediments were the most lethal followed by medium and fine. Gill tissues of exposed fish were examined histopathologically for evidence of injury or irritation at lethal and sub-lethal levels of suspended solids. Particle sizes of the three grades of sediment were compared with suspended sediments in water samples collected from the Fraser River.

At the time of writing this abstract tests are being prepared to expose adult sockeye salmon to suspended Fraser River sediments and the results will be reported at the workshop. Measurements of leucocytes and blood glucose are planned to assess stress levels.

QUELQUES EFFETS DES SÉDIMENTS EN SUSPENSION DE LA RIVIÈRE FRASER SUR LE SAUMON SOCKEYE

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Des saumons sockeye juvéniles furent exposés à des sédiments fins, moyens et à gros grains provenant de bancs de sable de la rivière Fraser. Des mesures de solides en suspension furent prises durant les bioassais afin de documenter les niveaux d'exposition. Les sédiments à gros grains se révélèrent les plus létaux, suivis des sédiments moyens et fins. Les tissus de branchies des poissons exposés furent examinés histopathologiquement afin de trouver évidence de blessures ou d'irritations à des niveaux létaux et sub-létaux de solides en suspension. Les grosseurs des particules des trois catégories de sédiment furent comparées avec les sédiments en suspension provenant d'échantillons d'eau collectés dans la rivière Fraser.

Au moment d'écrire ce résumé, des tests étaient en train d'être préparés pour exposer des saumons sockeye adultes à des sédiments de la rivière Fraser, et les résultats seront reportés durant l'atelier. Des mesures de leucocytes et de glucose dans le sang sont planifiées en vue d'estimer les niveaux de stress.

The Fraser River rises in the Rocky Mountains and flows through the interior of British Columbia to the Strait of Georgia. For natural reasons the Fraser River is highly turbid owing to suspended sediment for most of the year. Records of turbidity and non-filtrable residue at Hell's Gate in the Fraser Canyon about 205 km from the mouth indicate that maximum values occur with the onset of spring freshet. Suspended sediment decreases throughout the summer and autumn to a minimum in winter. The amount of suspended sediment varies from year to year and peaks occur when heavy rains or landslides occur.

Juvenile Pacific salmon (*Oncorhynchus* spp.) migrate seaward in the Fraser River in March, April and May and may encounter peak values of suspended sediment. Adult salmon generally encounter lesser amounts of suspended sediment since suspended sediment declines as summer progresses.

Under terms of a 1937 Convention the International Pacific Salmon Fisheries Commission is charged with protection, preservation and extension of Fraser River sockeye (*O. nerka*) and pink salmon (*O. gorbuscha*). Commission records indicate that from time to time there have been unexplained losses of adult Early Stuart sockeye during migration through 650 km of the Fraser River to the spawning grounds 1000 km from the mouth. Some data suggest a relationship between loss of Early Stuart sockeye and turbidity in the Fraser River but the relationship is inconclusive. There are no indications that sockeye smolts or pink fry, which migrate downstream in the Fraser in the spring, are adversely affected by suspended sediments, but on the other hand, the amount they can tolerate isn't known.

To examine possible effects of suspended sediments, juvenile and adult sockeye were exposed to Fraser River sediments in the laboratory. Studies commenced with tests of juvenile sockeye using sediments collected from sand bars in the Fraser River. For test purposes the sediments were graded into four components and juvenile sockeye were exposed to a series of concentrations. The results indicated coarse sediments were nearly ten times as toxic as fine sediments (Table 1).

Table 1.

Sediment (microns)	Acute Toxicity, 96 h LC_{50}, mg/L		
		Depth	
	Surface	(15 cm)	Mean
Fine (<74)	16,910	18,209	17,559
Intermediate-fine (74-149)	7,276	9,026	8,149
Intermediate-coarse (149-295)	4,847	4,904	4,875
Coarse (200-991)	1,465	1,883	1,674

Histological examination of gill tissue taken from juvenile sockeye exposed to 3000 mg/L fine sediments for four days indicated some swelling, separation of epithelium from pillar cells and necrosis. No mortalities occurred at 3000 mg/L.

Examination of suspended sediments collected from the Fraser River during upstream migration of Early Stuart sockeye in July 1983 indicated the particle size distribution was similar to that of the fine type sediment to which juvenile sockeye were exposed in the laboratory. Based on this analysis, adult Early Stuart sockeye were collected at Hell's Gate in early July 1984 and transported to the laboratory for subsequent exposure to fine sediments.

Sockeye were exposed to about 1500 mg/L of "fine" sediment for nine days. This amount of sediment was similar to the highest values measured in the Fraser River during summer sockeye migrations. No mortalities were attributed to suspended sediment during these tests. Leucocrits, hematocrits and blood glucose of experimental and control fish were compared. Leucocrits were not significantly different, but hematocrits were greater among experimentals than controls. Blood glucose, a stress indicator, was higher among experimental fish than controls but the difference was confirmed in only three of the four comparisons.

Complete reports of the foregoing tests will be reported subsequently. Further studies of the effects of suspended sediments are proposed.

A STUDY OF THE IMPACT OF TRIAZINE HERBICIDES UPON PERIPHYTIC ALGAE

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Triazines are extremely effective herbicides because of their ability to block the electron transport system of Photosystem II. Consequently, they are widely used in agricultural watersheds or directly in wetland management. There is, however, little quantitative information on the impact of such compounds on natural algal communities.

An investigation of the impact of simazine (2-chlor-4,6-bis[ethylamino]-S-triazine) and the more newly developed and as yet incompletely registered terbutryn (2-[tertbutylamino-]-4-[ethylamino]-6-[methyl thio]-S-triazine) upon algal communities in freshwater is reported. Rather than an examination of impact on phytoplankton, this is benthic algae which dominate shallow littoral waters most likely to receive such compounds.

Primary purposes were to investigate dose related impact upon primary productivity and algal biomass accumulation. Since the occurence of triazine resistance and its biochemical basis is known for some biotypes of higher plants, community structure change was also to be expected. Experiments were conducted in the shallow open waters of the 2,000 ha Delta Marsh on the southern shore of Lake Manitoba.

Experimental conditions involved the placement of cylindrical PVC enclosures into the shallow water. A number of 6.0 mm diameter extruded acrylic rods were inserted vertically into the sediment within each enclosure to act as substrata for periphyton colonization and growth. Substrata had been previously scored with a saw at pre-determined vertical intervals for ease of sampling. Enclosures were treated with 0.1, 1.0 and 5.0 mg/L reagent grade simazine and 0.01, 0.1 and 1.0 mg/L reagent grade terbutryn (Ciba Geigy). A 7th enclosure was maintained as control.

At regular intervals following herbicide addition, replicated segments of substratum were sampled for the estimation of periphyton carbon assimilation rate and chlorophyll content. Actual herbicide concentrations in each enclosure were also determined.

Inhibition of periphyton growth by both herbicides, with the degree of inhibition related to herbicide concentration is reported. EC_{50} values of between 0.1 and 1.0 mg/L for simazine and at approximately 0.01 mg/L for terbutryn were determined. Terbutryn appeared to exceed the toxicity of simazine by a factor of approximately 50.

Recovery of periphyton communities following the removal of herbicide (by flooding) is also reported. Although it was clear that treated and control enclosures were very similar in many ways, the presence of the herbicides did induce changes in several chemical parameters. Oxygen regimes were depressed and nutrients elevated. This suggested that an examination of the impact of such herbicides should incorporate the possibility of both direct and indirect effects. Principal component analysis did indeed show that herbicidal effects were related to altered nutrient regimes as well as herbicide concentration.

That altered nutrient regimes might be associated with herbicide activity at the sediment interface within enclosures was investigated by the addition of simazine to enclosures, some of which were open to the sediment (as used initially), but some of which were sealed from sediment contact by having bottoms placed into them. Over a period of time it was clear that in open-bottomed enclosures oxygen became depressed, while ammonia, phosphorous and silicon levels were elevated. The degree of depression of elevation was related to the concentration of added herbicide.

A suggested explanation of these phenomena is that herbicide had inhibited the photosynthetic epipelic community at the sediment surface, and that the resultant depressed oxygen levels stimulated sediment release of nutrients. Such a release might be considerably facilitated if the nutrient trapping capacity of the epipelic community had been inhibited by the herbicide. Elevated nutrient regimes and depressed oxygen levels could not be related to the death of enclosed macrophytes, for none were contained within the enclosures.

ÉTUDE DE L'IMPACT D'HERBICIDES À BASE DE TRIAZINE SUR LES ALGUES DU PÉRIPHYTON

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Les composés à base de triazine sont des herbicides extrêmement effectifs parce qu'ils peuvent bloquer le transport des électrons dans la photosynthèse de type II. Ils sont par conséquent beaucoup utilisés dans les bassins de régions agricoles ou directement dans l'aménagement des marais. Malgré cela, il n'existe que peu d'information quantitative sur l'impact que ces composés ont sur les communautés naturelles d'algues.

Nous reportons une étude sur l'impact du simazine (2-chloro-4,6-bi [éthylamino]-s-triazine) et du terbutryn (2-[tertbutylamino]-4-[éthylamino]-6-[méthylethio]-s-triazine), lequel a été développé récemment et n'est pas encore complètement enregistré, sur des communautés d'algues d'eau douce. Les algues benthiques dominent les eaux littorales peu profondes et sont par conséquent plus susceptibles à de tels composés que ne l'est le phytoplancton.

Notre but premier était d'évaluer l'impact de différentes doses sur la productivité primaire et sur l'accumulation dans la biomasse des algues. Etant donné que l'on sait que la résistance à la triazine existe dans certains biotypes de plantes supérieures et qu'on en connaît la base biochimique, on s'attendait aussi à un changement de structure dans la communauté. Des expériences furent conduites dans les eaux libres et peu profondes de Delta Marsh (2000 ha), sur la rive sud du lac Manitoba.

Les conditions expérimentales comprenaient la mise en place d'enclos cylindriques en CPV (chlorure de polyvinyle-PVC) en eau peu profonde. Plusieurs cylindres en acrylique, extrudés et ayant 6.0 mm de diamètre, furent insérés verticalement dans le substrat afin d'agir comme substrat pour la colonisation et la croissance du périphyton. Les cylindres avaient été préalablement marqués avec une scie à intervalles verticaux prédéterminés afin de faciliter l'échantillonnage. Les enclos furent traités avec 0.1, 1.0 et 5.1 mg/L de simazine et avec 0.01, 0.1 et 1.0 mg/L de terbutryn (Ciba Geigy). Un 7ème enclos fut gardé comme témoin.

Après que l'herbicide ait été ajouté, des segments dupliqués du substrat furent échantillonnés à intervalles réguliers en vue d'estimer le taux d'assimilation de carbone et le contenu en chlorophylle du périphyton. Les concentrations réelles en herbicide dans chaque enclos furent aussi déterminées.

Nous reportons l'inhibition de croissance du périphyton par les deux herbicides ainsi que le degré d'inhibition pouvant être relaté à la concentration de chaque herbicide. Nous avons déterminé des valeurs de CI_{50} entre 0.1 et 1.0 mg/L pour le simazine et d'approximativement 0.01 mg/L pour le terbutryn. Le terbutryn semble excéder la toxicité du simazine par un facteur d'environ 50.

Nous reportons aussi la récupération des communautés de périphyton après que les herbicides aient été enlevés (par inondation). Bien que les enclos traités et témoins fussent très similaires, la présence des herbicides produisit des changements réels dans plusieurs paramètres chimiques. La concentration d'oxygène diminua et les matières nutritives augmentèrent. Ceci suggère qu'un examen de l'impact de tels herbicides devrait inclure la possibilité d'effets directs et indirects. L'analyse des composantes principales révéla en effet que les effets des herbicides étaient autant reliés à l'altération des cycles nutritifs qu'à la concentration des herbicides.

On étudia la possibilité que l'altération des cycles nutritifs soit associée à l'activité des herbicides à l'interface des sédiments à l'intérieur des enclos, en ajoutant du simazine à des enclos à fond ouvert (tels qu'utilisés initialement) et à d'autres à fond scellé. Il devint clair qu'après un certain temps l'oxygène des enclos à fond ouvert diminuait tandis que les niveaux d'ammoniac, de phosphore et de silicium augmentaient. Le degré de diminution ou d'augmentation était relié à la concentration d'herbicide ajouté.

Une explication possible de ces phénomènes est que l'herbicide inhibe la communauté photosynthétique à la surface des sédiments, et la baisse en oxygène qui en résulte stimule le relâchement d'éléments nutritifs par les sédiments. Un tel relâchement peut être facilité considérablement si la capacité de rétention d'éléments nutritifs de la communauté de surface des sédiments est inhibée par l'herbicide. Les hautes concentrations d'éléments nutritifs et les bas niveaux d'oxygène ne peuvent être tenus responsables de la mort des macrophytes étant donné qu'il n'y en avait aucun dans les enclos.

SIMULTANEOUS INTEGRATED ECOTOXICOLOGICAL EVALUATION

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A practical and economical approach to identify and measure ecotoxicity of waste waters.

When one wishes to identify and measure the ecotoxic impact of an unknown discharge, a scan of microbial, chemical and ecotoxicological parameters must be used for a first estimation of this potential threat.

A practical yet economical methodological approach using three operational steps is suggested. To better understand and appreciate this potential impact, ecotoxicity is conceptualized as progressing spirally. This spiral progresses through three successive zones corresponding respectively to the local, regional and global centers of impact. Two perpendicularly opposed axes separate these zones and distinguish between lethal versus sublethal effects and stable versus chronic ecotoxicities.

ÉVALUATION ÉCOTOXICOLOGIQUE INTÉGRÉE SIMULTANÉE SELON UN CONCEPT DE LA SPIRALE DE L'ÉCOTOXICITÉ

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Toute évaluation écotoxicologique d'un rejet doit être faite avec une gamme suffisamment étendue de paramètres chimiques, microbiologiques et ecotoxicologiques afin d'obtenir une première estimation de ce fléau potentiel.

Une approche pratique et économique faisant appel à trois étapes opérationnelles est suggérée. Pour mieux expliciter la novicité du rejet, cette approche fait appel à une conceptualisation représentant la progression "spiralée" de l'écotoxicité.

Cette spirale s'ouvre en 3 portions successives qui correspondent à 3 niveaux de protection environnementale où l'impact du rejet est considéré comme étant respectivement local, régional et global. Deux axes perpendiculairement opposés, séparent ces niveaux et distinguent les écotoxicités létales et sous-létales, d'une part, et les effets stables versus chroniques, d'autre part.

INTRODUCTION

In the last two decades a number of environmental disasters (mercury in Minimata, Japan, dioxins in Seveso, Italy, contaminants in the Love Canal, United States, acid rain and so on) have heightened public awareness of the effect of toxic waste on the environment.

Increased public concern, as an outcome of news media and pressure groups, and new legislation aimed a environmental protection has gradually spurred more research on various ecotoxicological phenomena as well as the improvement of industrial projects and programs to restore the aquatic environment.

In this context, persons in charge of monitoring the environment are aware that the information disseminated to the public will be of prime importance in gradually reducing pollution. There will undoubtedly be more and more information available owing to new policies allowing freer access to government files.

But informing the public of often complex environmental problems is by no means simple; there are occasionally a number of different positions on a single issue.

Faced with these conflicting interpretations, some people will find it easier to accept alarmist views, convinced that the truth has now been revealed.

The public then adopts a relatively simplistic, even sensationalist, approach, and cannot appreciate all the aspects of a problem. Opinion-makers are not the only ones responsible for this state of affairs; some of the blame can be laid at the door of the environmental managers and scientists concerned.

It must be recognized, however, that the latter have few ways of illustrating complex environmental issues and promoting the dissemination of balanced information.

The need for means of depicting the problem of ecotoxicity led us to search for a new, integrated representation of its multiple affects; accordingly, we opted for a spiral form, which fully and yet simply explains the phenomenon.

Spiral representation of the various effects of ecotoxicity

Figure 1 shows this spiral, with its three levels of environmental protection, separated by two perpendicular axes.

The first axis separates lethal versus sublethal effects, while the second separates directly acting phenomena, with acute to subacute toxic effects, from insidiously acting phenomena leading to chronic toxic effects.

The two axes allow us to define three levels, whose consequences on the aquatic environment are shown in Table 1.

Table 2 provides three examples of the effects of toxic waste released into the aquatic environment, in order to make Table 1 easier to understand.

The spiral concept of ecotoxicity means that we must consider not only the

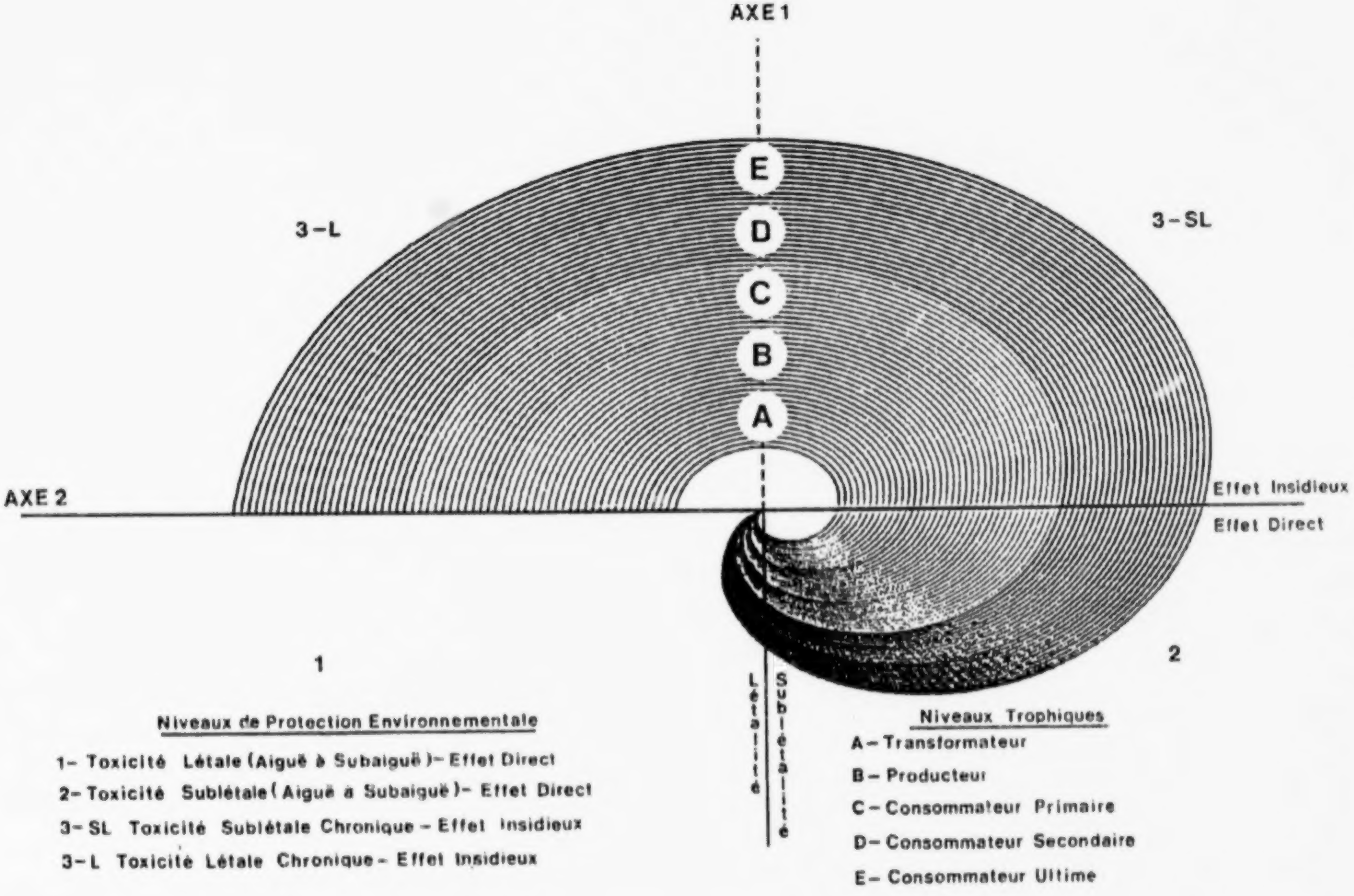

AXE 1
E
D
C
B
A
3-L
3-SL
AXE 2
Effet Insidieux
Effet Direct
1
2
Létalité
Sublétalité
Niveaux de Protection Environnementale
1- Toxicité Létale (Aiguë à Subaiguë)- Effet Direct
2- Toxicité Sublétale (Aiguë à Subaiguë)- Effet Direct
3- SL Toxicité Sublétale Chronique - Effet Insidieux
3- L Toxicité Létale Chronique - Effet Insidieux
Niveaux Trophiques
A - Transformateur
B - Producteur
C - Consommateur Primaire
D - Consommateur Secondaire
E - Consommateur Ultime

FIGURE I SPIRALE DE L'ECOTOXICITE .

Table 1. Three levels of environmental protection for aquatic toxicity.

Level	Ecotoxic impact		Environmental degradation		Suggested correction
	Level of toxicity	Scope	Cause	Consequences	
first	Acute to subacute lethal toxicity	Local	Excessive presence of pollutants (contaminants, pathogenic agents, disturbed physico-chemical conditions, etc.)	Nearly complete disappearance of biotic components of the environment, with the exception of ultra-tolerant species, (eg., certain bacteria)	Primary treatment of waste
second	Acute to subacute sublethal toxicity resulting from easily quantifiable directly acting phenomena	Regional	Sublethal pollution causing an unbalance in the aquatic environment; these substances may be biodegradable, but not bioaccumulable hence safely assimilable	Disturbance of biological diversity, and physiological stress of specific duration	Secondary, biological treatment of wastewater, considering the assimilative capacity of the receiving environment
third	Chronic toxicity resulting from insidiously and dynamically acting phenomena, relatively difficult to quantify	Global	Insidious toxicity, often difficult to assess, not safely assimilated by the receiving environment, and acts through mechanisms such as bioaccumulation and/or genotoxicity	Reduction in long-term survival, growth rate and reproduction of species; destabilization of structures and functions of aquatic ecosystems, with possible recurrence of lethal effects; introduction of genotoxic problems	Elimination of pollutants at source, following risk and hazard assessment taking into account socio-economic considerations

Table 2. Possible toxic effects of three different incidents, with and without treatment of waste.

<table>
<tr><th>Toxic substances released</th><th>Without treatment</th><th>Primary treatment (eg; neutralization of pH and removal of precipitate)</th><th>Secondary treatment (eg., biological treatment and removal of sludge)</th></tr>
<tr><td>H_2SO_4</td><td>• acute lethal toxicity at high concentration (1000 mg/L; Nriagu, 1978)
• acute sublethal toxicity (10 mg/L; Nriagu, 1978)
• insidious toxicity, because of possibility of increased bioavailability of metals in the environment due to change in pH favoring bioaccumulation</td><td>• possibility of lingering weak sublethal toxicity, relatively easily assimilated by the receiving environment
• precipitate to be recycled</td><td>Useless for this type of pollutant</td></tr>
<tr><td>Phenol</td><td colspan="2">• acute lethal toxicity at high concentration (10 mg/L; EPA, 1981)
• acute sublethal toxicity at weak concentration (2000 to 10 μg/L; EPA, 1981)
• insidious toxicity, because of possibility of formation of chlorophenols whose bioaccumulation will have organoleptic and possibly mutagenic consequences</td><td>• possibility of lingering weak sublethal toxicity relatively easily assimilated
• biological sludge to be incinerated, buried or digested</td></tr>
<tr><td>PCB (Polychlorobiphenyls)</td><td colspan="3">• PCBs are toxic at very low levels; 10 to 100 μg/L can produce lethal effects, 1 to 10 μg/L affect reproduction, and 0.01 to 0.1 μg/L are noxious, owing to bioaccumulation (Roberts et al., 1979).
• Secondary treatment can be effective to concentrate the PCBs in biological sludge. However, the environmental problem remains, since PCBs are not easily biodegraded.
• Elimination at source is the only effective way to control these pollutants.</td></tr>
</table>

concentration of the toxic substance, but also factors such as the scope of its toxicity, its persistence, and the likelihood of transformation and/or bioaccumulation.

Since this spiral representation of ecotoxicity is only a concept, it must be tested, like any other scientific concept, to determine whether it is relatively simple to apply, at an acceptable cost.

Our approach has been tested with waste from other dumps, allowing us to determine a practical means of applying it (see Sections 3 and 4 of the original text).

Since the beginning of the 1984-85 fiscal year, the Environmental Protection Service, Quebec Region, has been evaluating this approach (Figures 2 and 3) as a practical and economical detection tool. The purpose of this method is to assess the main ecotoxicological problems which may be caused by the release of various liquid wastes whose ecotoxicity is poorly known. This publication is the latest phase in our process of combining the suggested approach with the ecotoxicity spiral. It is part of recent efforts to develop overall measures to assess the ecotoxicological risks of a waste substance or new product (Corn et al., 1982; Katsuoshi, 1982).

CONCLUSION

The noxiousness of waste substances can be better understood if we think of ecotoxicity as a spiral. The spiral progresses through three successive zones, corresponding respectively to the local, regional and global centres of impact.

Two perpendicular axes separate these zones and distinguish between lethal versus sublethal effects and, direct versus insidious toxic phenomena leading to chronic effects (see Figure 1).

Using the spiral we can examine the different aspects of a substance's noxiousness; it provides a complete and simple illustration of the risks attendant on the substance so as to inform the public properly.

The concept of the ecotoxicity spiral has been tested with success for four wastewaters from dumps, using an analytical program based on the three levels of ecotoxicity.

The results obtained show that:

[i] any ecotoxicological assessment of a waste material must be done with a sufficiently wide range of chemical, microbiological and ecotoxicological parameters, so as to ensure that its ecotoxicity is accurately estimated.

[ii] the method adopted involves a fairly complete integrated description of the noxiousness of waste materials, since it specifies not only the chemical and microbiological agents, but also the substance's lethal, sublethal and chronic effects.

We can recommend an operational procedure, based on this testing procedure.

The approach suggested is detailed in Table 3, and illustrated in Figures 2 and 3. In short, to estimate the major environmental problems, an ecotoxicological assessment on 10 samples of a wastewater is done as follows:

First operational step:

- essential activity; cost of 30 person-days
- purpose : to identify probable ecotoxic problems
- performance: information obtained in less than six days, following initial sampling sequence

Second operational step:

- only if necessary; cost of 3 to 40 person-days and possibly $6K
- purpose : to determine the nature and scope of the problems detected
- performance: information obtained in less than 20 days, following initial sampling sequence

Third operational step:

- only if necessary; cost of 5 to 170 person-days and possibly $13K
- purpose : to examine in greater depth the nature and scope of ecotoxicity, by using biotests conducted at higher levels of biological organization and/or conducting costly chemical analysis of non-preservable substances
- performance: information obtained in less than 40 days, following a second sampling sequence

FIGURE 2

SIMULTANEOUS INTEGRATED ECOTOXICOLOGICAL ASSESSMENT OF WASTEWATER

OPERATIONAL STEPS FOLLOWING THE INITIAL SAMPLING SEQUENCE (35L/SAMPLE)

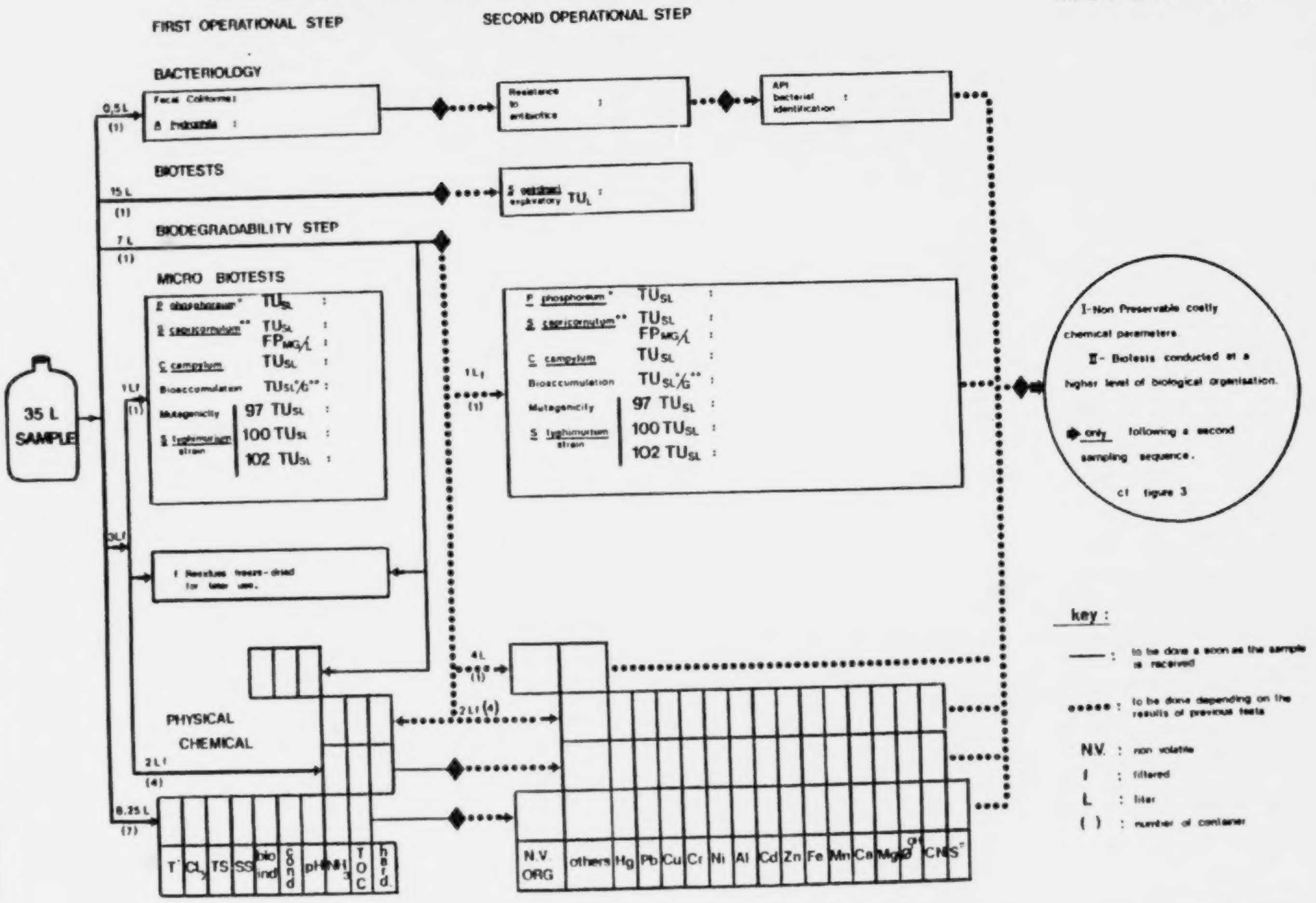
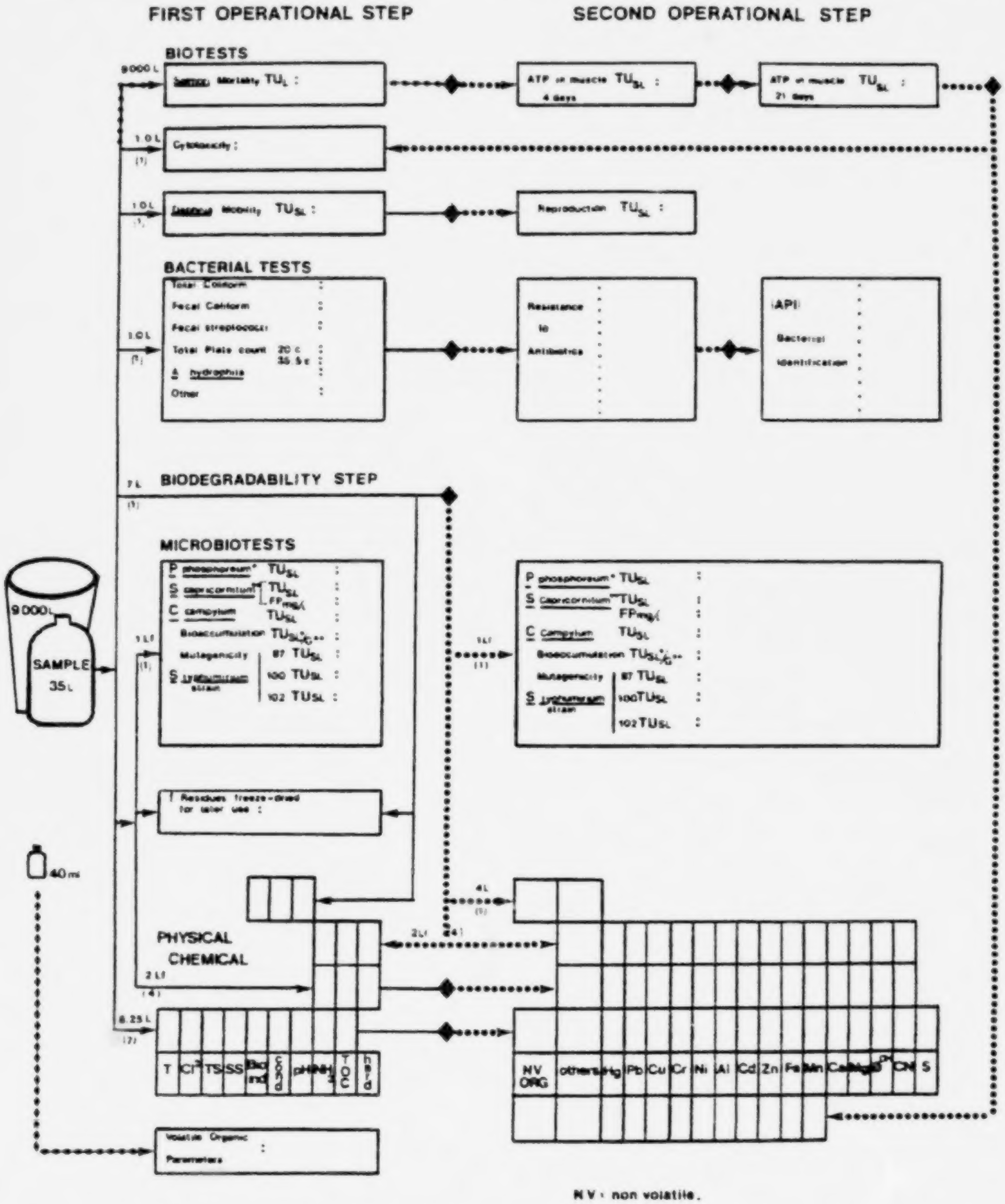

THIRD OPERATIONAL STEP
FIRST OPERATIONAL STEP
SECOND OPERATIONAL STEP
BACTERIOLOGY
0,5 L (1)
Fecal Coliforms :
A. hydrophila :
Resistance to antibiotics :
API bacterial identification :
BIOTESTS
15 L (1)
exploratory TU_L :
BIODEGRADABILITY STEP
7 L (1)
MICRO BIOTESTS
P. phosphoreum* TU_SL :
S. capricornutum** TU_SL : FP_MG/L :
C. campylum TU_SL :
Bioaccumulation TU_SL*/G** :
Mutagenicity S. typhimurium strain 97 TU_SL : 100 TU_SL : 102 TU_SL :
1 L f (1)
1 L f (1)
3 L f
f Residues freeze-dried for later use.
35 L SAMPLE
4 L (1)
2 L f (4)
2 L f (4)
0.25 L (7)
PHYSICAL CHEMICAL
T° Cl2 TS SS bio ind cond pH NH3 TOC hard.
N.V. ORG others Hg Pb Cu Cr Ni Al Cd Zn Fe Mn Ca Mg Cl⁻ CN⁻ S⁼
I- Non Preservable costly chemical parameters.
II- Biotests conducted at a higher level of biological organisation.
only following a second sampling sequence.
cf figure 3
key :
—— : to be done a soon as the sample is received
••••• : to be done depending on the results of previous tests
N.V. : non volatile
f : filtered
L : liter
( ) : number of container

FIGURE 3

SIMULTANEOUS INTEGRATED ECOTOXICOLOGICAL ASSESSMENT OF WASTEWATER.

OPERATIONAL STEP FOLLOWING A SECOND SAMPLING SEQUENCE (40mL ~~AND~~/OR 35L TO 9000 L).


FIRST OPERATIONAL STEP
SECOND OPERATIONAL STEP
BIOTESTS
9000 L
Salmon Mortality TU_L :
ATP in muscle TU_{SL} : 4 days
ATP in muscle TU_{SL} : 21 days
1.0 L (1)
Cytotoxicity :
1.0 L (1)
Daphnia Mobility TU_{SL} :
Reproduction TU_{SL} :
BACTERIAL TESTS
1.0 L (1)
Total Coliform :
Fecal Coliform :
Fecal streptococci :
Total Plate count 20 c 35.5 c :
A. hydrophila :
Other :
Resistance to Antibiotics
(API) Bacterial identification
7 L (1)
BIODEGRADABILITY STEP
MICROBIOTESTS
P. phosphoreum* TU_{SL} :
S. capricornutum** TU_{SL} : $FP_{mg/l}$:
C. campylum TU_{SL} :
Bioaccumulation TU_{SL} :
Mutagenicity 87 TU_{SL} :
S. typhimurium strain 100 TU_{SL} : 102 TU_{SL} :
1 Lf (1)
9000 L
SAMPLE 35 L
Residues freeze-dried for later use :
40 ml
4 L (1)
2 Lf (4)
PHYSICAL CHEMICAL
2 Lf (4)
6.25 L (7)
T
Cl
TS
SS
Bio ind
cond
pH
NH3
TOC
hard
NV ORG
others
Hg
Pb
Cu
Cr
Ni
Al
Cd
Zn
Fe
Mn
Ca
Mg
CN
S
Volatile Organic Parameters :

N V : non volatile.

f : filtered.

L : Liter.

( ) : number of containers.

◆ : decision point.

KEY: ——— To be done a soon as the sample is received.

–•–•–• Special sampling procedure required.

•••••• To be done depending on the results of previous tests.

Table 3. Operational procedure for a global ecotoxicological assessment.

Operations, analyses or bioassays	Level of environmental protection	Pertinence and/or levels of ecotoxicity	Volume required per sample*	Methodological references	Cost**
[A] FIRST OPERATIONAL STEP (see Figure 2).					
A-1 Sampling No. 1, including in situ analyses (pH, T, etc.)	1-2	Basic activity	N/A	Environment Canada 1979 & 1980	8.0 p-d
A-2 Distribution and routing of subsamples, filtration and freeze-drying of residues	N/A	In preparation for subsequent steps.	N/A	Environment Canada 1979 & 1981	1.0 p-d
A-3 Microbiological analyses • Fecal coliforms • Aeronomas hydrophila	1,2	Human pathogen Fish pathogen	0.5 L	APHA et al, 1980 Shotts & Rimler, 1973	1.5 p-d
A-4 Biodegradability 5 d	2,3	Chronic: persistence	7.0 L	van Coillie et al., 1983	1.0 p-d
A-5 Screening bioassays: •Bacteria - bioluminescence 5 min. Photobacterium phosphoreum	1***,2	Lethal to acute sublethal	1.0 L f	Beckman, 1980	1.5 p-d
•Algae - growth 4 d Selenastrum capricornutum.	2	Acute to subacute sublethal		Blaise et al., 1982	1.0 p-d
•Protozoa ingestion 1 d Colpidium campylum	2	Acute to subacute sublethal		Dive & Leclerc, 1975	1.5 p-d
A-6 Accumulation of ecotoxicity in algae over 24 hours	3	Chronic: bioaccumulation		Blaise et al., 1982	5.0 p-d
A-7 Genotoxicity bioassays: Mutagenicity test	3	Chronic: mutagenicity		Ames et al., 1975	5.0 p-d
A-8 General physico-chemical analyses: •Ammonia •Total organic carbon	1,2	Essential parameters to determine significance of toxic effects	2.5 L and 2.0 L f	APHA et al., 1980	3.0 p-d

Table 3 cont...

Operations, analyses or bioassays	Level of environmental protection	Pertinence and/or levels of ecotoxicity	Volume required per sample*	Methodological references	Cost**
A-8 cont...					
•Hardness •Total and suspended solids					
A-9 Initial assessment	1,2,3	Co-ordination and decision concerning further tests or report	N/A	N/A	1.5 p-d
Sub-total A	1,2,3		13 L		30 p-d
[B] SECOND OPERATIONAL STEP (See Figure 2)					
B-1 Sampling included in A-1					
B-2 Identification of pathogenic agents and assessment of their resistance to antibiotics	1,2,3	Specify extent of microbial problem	N/A	Washington et al., 1971	0 to 5 p-d
B-3 Assessment of toxicity on samples after 5 d of biodegradation	1,2,3	Check persistence of lethal and/or chronic ecotoxicity identified	1.0 L f Included in A4	See A 5 to A	1 to 16 p-d
B-4 Chemical dosage of contaminants after standard preservation: •Heavy metals •Mercury •Cyanide •Sulphide •Phenols •Non-volatile organic substances •Others	1,2,3	Verify and identify chemical causes of ecotoxicity identified	5.75 L 4.0 L and 2.0 L f Included in A4	APHA et. al., 1980	1 to 17 p-d and possibly $6K

Table 3 cont...

Operations, analyses or bioassays	Level of environmental protection	Pertinence and/or levels of ecotoxicity	Volume required per sample*	Methodological references	Cost**
B-5 Complementary assessment	1,2,3	Co-ordination and decision on further activity or report	N/A	N/A	1 to 2 p-d
B-6 Exploratory evaluation of lethal toxicity on fish	1	Acute lethal	15 L		0 to 3 p-d
Subtotal B	1,2,3		20.75 L		30 to 40 p-d and possibly $6K

[C] THIRD OPERATIONAL STEP.

A second sampling sequence can be planned and carried out as necessary to obtain the results of bioassays conducted at higher biological levels or to acquire pertinent information from non-preservable, costly chemical parameters such as volatile organic substances. During the second sampling sequence, the activities described in the first and second operational steps may be repeated if necessary (see Figure 3).

Operations, analyses or bioassays	Level of environmental protection	Pertinence and/or levels of ecotoxicity	Volume required per sample*	Methodological references	Cost**
C-1 Sampling 2	1,2	Basic activity	N/A	see A-1	1 to 13 p-d
C-2 Activities described in first step	1,2,3	As necessary only	0 to 13 L	see A2 to A8	0 to 22 p-d
C-3 Activities described in second step	1,2,3	As necessary only	0 to 20.75 L	see B2 to B4	0 to 40 p-d
C-4 Ecotoxicological bioassays at higher biological levels:					
· Salmo gairdneri mortality 4 d	1	Acute lethal	400 L to 9000 L	Environment Canada, 1980b Blaise, 1984	0 to 25 p-d
muscular ATP 4 d	2	Acute sublethal			0 to 15 p-d
muscular ATP	2,3	Subacute sublethal leading to chronic			0 to 35 p-d
bioaccumulation and cytotoxicity 21 d	2 - 3				
· Daphnia pulex mobility	2	Acute sublethal	1 L	Environment Canada, 1979b	0 to 5 p-d
reproduction	3	Chronic			0 to 10 p-d

Table 3 cont...

Operations, analyses or bioassays	Level of environmental protection	Pertinence and/or levels of ecotoxicity	Volume required per sample*	Methodological references	Cost**
C-5 Carcinogenicity bioassays Test with mammal or cytotoxicity	3	Chronic	10 L		possibly $10K
C-6 Determination with costly, non-preservable chemicals •Volatile organic parameters	1,2		40 ml	Federal Register, 1979	possibly $3K
C-7 Report	1,2,3		N/A	N/A	1 to 5 p-d
Subtotal C	1,2,3		40 ml to 9000 L		5 to 170 p-d and possibly $13K

*Volume per sample: the sign (f) indicates that it must be filtered at 0.22µm, following which the filtrate and residue may be freeze-dried for later analysis.

**Cost of 10 samples in p-d (person-days) and/or in financial support (K=$1000).

***The EC_{50} (Effective concentration at 50%) determined with Photobacterium phosphoreum (parameter: bioluminescence in 5 minutes) for various products and effluents is often fairly close to their LC_{50} (Lethal concentration at 50%) determined with Salmo gairdneri (parameter: cumulative mortality in 96 hours); this has often been proven in the EPS laboratories in Longueuil (unpublished data). It follows that the bioassay with Photobacterium phosphoreum can not only reveal acute sublethal ecotoxicity but is also useful in estimating lethal ecotoxicity.

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CONTRIBUTED ABSTRACTS

ECOTOXICOLOGICAL ASSESSMENT OF PULP AND PAPER EFFLUENTS

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Comparative biological indicator data, gathered in recent years, are presented specifically as they relate to pulp and paper effluents. Bacterial (Microtox, Fluctuation test), algal (*Selenastrum capricornutum*) and fish (lethal and sublethal tests with *Salmo gairdneri*) bioassay results indicate clearly that all effluents are not created equal because of notable differences in inter- and intra-specific toxicity responses. While most of the effluents tested were deleterious to test organisms, the extent of the toxicity response was dependent upon the indicator used, the mill technology employed, as well as on the wastewater treatment applied. The use of the Microtox and algal bioassays appear to be promising tools for toxicity screening of pulp and paper effluents.

ÉVALUATION ÉCOTOXICOLOGIQUE DES EFFLUENTS DE PÂTES ET PAPIER

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Des données comparatives basées sur des indicateurs biologiques et collectées dans les dernières années, sont présentées en relation avec les effluents de pâtes et papier. Des résultats d'essais biologiques menés avec des bactéries (Microtox, test de fluctuation), des algues (*Selenastrum capricornutum*) et des poissons (tests létaux et non létaux menés avec *Salmo gairdneri*) indiquent clairement que tous les effluents ne sont pas créés égaux, et ceci à cause de claires différences dans les réponses de toxicité inter- et intra-spécifiques. Bien que la plupart des effluents examinés étaient néfastes aux organismes étudiés, l'ampleur des résultats de toxicité dépendait de l'indicateur utilisé, de la technologie employée au moulin et aussi du type de traitement des eaux usées. L'utilisation de Microtox et des essais biologiques avec algues se révèlent des outils prometteurs dans les études de toxicité sur les effluents de pâtes et papier.

PREDICTING CONTAMINANT EFFECTS ON WHOLE LAKE ECOSYSTEMS

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The structure of pelagic ecosystems, and to some degree benthic systems as well, is dominated by processes related to body size. Primary production in the oceans and large lakes is mainly by microscopic algae. Herbivores are small and most predators feed on prey considerably smaller than themselves. Body size is therefore a rough indication of trophic level and the structure of pelagic ecosystems can be described in terms of a biomass size spectrum. The shape of the spectrum is a function of biomass conversion efficiencies, predator-prey size ratios and the allometric relationship between growth rates and body size. The total biomass is a function of total nutrient loads. Since the biomass size spectrum, on a gross scale, is predictable from relatively few variables, it is possible to predict changes in the shape of this spectrum after addition of toxic chemicals. The necessary data can be obtained from laboratory experiments on contaminant effects on growth rates and conversion efficiencies. It is therefore possible to predict the effect of toxic contaminants on biomass and production of any trophic level in the ecosystems of large lakes if the contaminant affects most trophic levels to a similar degree. As an example, this technique is used to predict the impact of a representative toxicant on the pelagic ecosystem of Lake Ontario.

PRÉDICTION DES EFFETS DE CONTAMINATION SUR LES ÉCOSYSTÈMES LACUSTRES

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La structure des écosystèmes pélagiques, et aussi jusqu'à un certain point celle des systèmes benthiques, est dominée par des processus relatés à la dimension corporelle des organismes. La production primaire dans l'océan et dans les grands lacs s'effectue principalement à travers les algues microscopiques. Les herbivores sont petits et la plupart des prédateurs se nourrissent sur des proies considérablement plus petites qu'eux-mêmes. La grosseur des organismes fournit par conséquent une approximation du niveau trophique, et la structure des écosystèmes pélagiques peut être décrite en termes de variations dans la dimension de la biomasse. La forme de ces variations est en fonction de l'efficacité de conversion en biomasse, du rapport des dimensions prédateur-proie et de la relation allométrique entre la vitesse de croissance et la dimension corporelle. La biomasse totale est en fonction de la quantité totale des éléments nutritifs. Puisque les variations dans la dimension de la biomasse sont prévisibles selon une échelle approximative, il est possible de prédire des variations dans la forme des variations après l'addition de produits chimiques toxiques à partir de relativement peu de variables. Les données nécessaires peuvent être obtenues à partir d'expériences en laboratoire sur les effets des agents contaminants sur la vitesse de croissance et sur l'efficacité de conversion. Il est par conséquent possible de prédire l'effet des agents contaminants toxiques sur la biomasse et sur la production à n'importe quel niveau trophique dans l'écosystème des grands lacs, si l'agent contaminant affecte la plupart des niveaux trophiques au même degré. Comme exemple, cette technique est utilisée pour prédire l'impact d'un produit toxique représentatif sur l'écosystème pélagique du lac Ontario.

SPECIATION OF CADMIUM, ZINC AND COPPER IN CONTAMINATED AND UNCONTAMINATED AMERICAN LOBSTER (HOMARUS AMERICANUS) DIGESTIVE GLAND

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A rapid size-exclusion chromatographic-flame atomic absorption spectrophotometric method has been developed to distinguish between free metal and bound metal in lobster digestive gland extracts. The method is based upon a desalting procedure using a 100-10,000 molecular weight gel permeation material. In the lobster digestive gland extract the bound metal elutes after 4.3 minutes and the free metal after 6.2 minutes at an eluent flow rate of 3 ml/min. One determination takes 10-15 minutes. The technique has been applied to determine bound and "free" forms of cadmium, zinc and copper in lobster digestive gland extracts stored for some time without protease inhibitor, phenyl-methyl sulfonyl fluoride (PMSF), to generate "free" cadmium and zinc, and the chromatographic results obtained for "free" cadmium and zinc compared with that determined by polarography. The two methods gave equivalent results. Use of the protease inhibitor added to the digestive gland prior to work-up prevented the generation of "free" metal ions.

SPÉCIATION DE CADMIUM, ZINC ET CUIVRE DANS LES GLANDES DIGESTIVES CONTAMINÉES ET NON CONTAMINÉES DE HOMARDS AMÉRICAINS (HOMARUS AMERICANUS)

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Une méthode rapide de spectrophotométrie d'absorption atomique (chromatographie par flamme) et d'exclusion par grosseur a été développée pour distinguer les métaux libres des métaux liés dans des extraits de glande digestive de homards. La méthode est basée sur une procédure de dessalage utilisant un gel de poids moléculaire 100-10,000. Dans l'extrait de glande digestive de homards, le métal lié se décante après 4.3 minutes et le métal libre après 6.2 minutes, à une vitesse d'écoulement de 3 ml/min. Une détermination nécessite de 10 à 15 minutes. Cette technique fut utilisée pour déterminer les formes de cadmium, zinc et cuivre libres et liées dans des extraits de glande digestive de homards ayant été conservés pour quelque temps sans inhibiteur de protéase (fluorure de phényle-méthyle sulfonyle (FPMS)), et pour générer le cadmium et zinc 'libres'. Les résultats chromatographiques obtenus pour le cadmium et pour le zinc 'libres' furent comparés avec ceux déterminés par polarographie. Les deux méthodes produisent des résultats équivalents. L'emploi de l'inhibiteur de protéase ajouté à la glande digestive avant le test prévient la formation d'ions de métal 'libres'.

BIOASSESSMENT OF SEDIMENTS: PROTOCOL VIEW AND RECOMMENDED APPROACH

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Bioassay testing of sediments provides an integrated picture of potential toxic effects of chemical constituents. Consideration of physical/chemical partitioning of compounds between particulates and water plus an appreciation of a range of modes of toxic action of various chemicals provides the initial framework for test organism selection. Recognition of the different levels of ecosystem protection supports a test approach utilizing 3 environmental compartments. Review of documented sediment test methods identifies which systems and organisms appear most productive and informative.

A tiered bioassessment protocol utilizing elutriate, water-sediment interface and sediment phases for exposure of *Daphnia*, *Gammarus*, *Hexagenia* and fish would provide information to protect against acute lethality, reproductive impairment and accumulation of contaminants in aquatic biota as a result of dredging operations.

ESTIMATION BIOLOGIQUE DE SÉDIMENTS: PROTOCOLE ET APPROCHE RECOMMANDÉE

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L'analyse biologique des sédiments résulte en une vue d'ensemble des effets toxiques potentiels des constituants chimiques. La première étape de la sélection d'organismes à étudier inclut la considération du type de partitionnement physique/chimique des composés entre les composés particulaires et l'eau, plus une appréciation de plusieurs modes d'action toxique de divers composés chimiques. La reconnaissance des différents niveaux de protection de l'écosystème sous-entend une approche analytique utilisant 3 compartiments environnementaux. Une revue des méthodes d'analyse de sédiments documentées identifie les systèmes et organismes apparaissant les plus productifs et instructifs.

Un protocole d'estimation biologique tripartite utilisant la séparation par décantation, l'interface eau-sédiments et des phases de sédiments pour l'exposition de *Daphnia*, *Gammarus*, *Hexagenia* et de poissons, devrait fournir l'information nécessaire à la protection contre une mortalité aigüe, un détériorement de la reproduction et l'accumulation d'agents contaminants dans le milieu aquatique résultant d'opérations de drague.

A POTENTIAL IN SITU FISH BIOASSAY SAMPLER FOR LARGE RIVERS - MEASURING CORTICOSTEROID STRESS DUE TO SAMPLER CONFINEMENT

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Coho (*Oncorhynchus kisutch*) juveniles were held under flow-through conditions in an *in situ* sampler at loading densities of approximately 12 g/L. During a 14-day period of exposure the confined fish had a mean cortisol concentration of 47.06 ng/ml (range: 34.06-81.53) while control fish in the rearing pond had a mean concentration of 29.24 ng/ml (range: 7.31-74.73). Preliminary results are being evaluated in terms of sampler suitability for acute and subacute toxicity studies in large rivers.

UN ÉCHANTILLONNEUR POTENTIEL POUR LES BIOASSAIS AVEC POISSONS IN SITU POUR DES GRANDES RIVIÈRES - MESURE DU STRESS DÛ AU CONFINEMENT DANS L'ÉCHANTILLONNEUR PAR LES CORTICOSTÉROÏDES

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Des saumons coho juvéniles (*Oncorhynchus kisutch*) furent gardés sous des conditions de courant continu dans un échantillonneur *in situ* à des densités d'approximativement 12 g/L. Durant une période d'exposition de 14 jours, les poissons confinés avaient une concentration moyenne de cortisol de 47.06 ng/ml (variation: 34.06-81.53) tandis que des poissons témoins gardés dans un étang d'élevage avaient une concentration moyenne de 29.24 ng/ml (variation: 7.31-74.73). Les résultats préliminaires sont évalués en termes de la convenance de l'échantillonneur pour des études de toxicité aiguë et modérée dans de grandes rivières.

LABORATORY TEST PROCEDURES FOR ASSESSING THE ACUTE AND CHRONIC EFFECTS TOWARD SALMONID FISH CAUSED BY THE BRUSH CONTROL HERBICIDE KRENITE

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A variety of lethal and sublethal toxicity tests were used to evaluate the extent to which aerial spraying of Krenite (ammonium ethyl carbamoyl phosphonate; Dupont Canada Inc.), a conifer-release brush control herbicide, may pose a threat to resident or migratory salmonid fish. The laboratory bioassays were designed to examine the influence of differing environmental conditions (including receiving-water quality) on the tolerance of these fish to Krenite, and to determine the immediate and long-term adverse effects of dosing differing life stages. Threshold concentrations of this herbicide which caused stress reactions and effected avoidance/preference responses of underyearling salmonid fish were determined as part of this investigation.

The relative tolerance to Krenite for eight life stages of coho salmon (newly-fertilized egg through smolt) and five life stages of rainbow trout (newly-fertilized egg through fingerling) was determined by 4-day and 12-day median lethal concentration (LC_{50}) bioassays. Groups of fish surviving exposure to dilute strengths of Krenite at each of the early life stages were reared to fingerlings in uncontaminated freshwater. These fish were monitored for post-exposure survival, time to hatching, hatching success, time to yolk absorption, growth, behavioural and developmental anomalies. Additionally, their subsequent acute lethal tolerance to a second challenge with Krenite or to the reference toxicant pentachlorophenol was determined. Coho salmon pre-smolts and smolts surviving shortterm exposure to Krenite were examined for post-exposure mortalities in freshwater and seawater.

Controlled-exposure studies were conducted with underyearling rainbow trout and coho salmon in order to determine the influence of a number of environmental variables on their acute lethal tolerance to Krenite. These studies included an examination of the influence of receiving-water (diluent-water) characteristics, pH, test and acclimation temperature on LC_{50} values. The effects on LC_{50} values of aging of Krenite in freshwater, fish-loading density in bioassays, and static vs replacement bioassays, were also ascertained.

The median effective concentrations (EC_{50}) of Krenite which caused declines in numbers of circulating white blood cells (measured as leucocrit values) and numbers of circulating red blood cells (measured as hematocrit) in fingerling coho salmon were determined. These responses were interpreted as the threshold strengths of Krenite which were acutely stressful to this life stage of salmonid fish. A sharp gradient avoidance/preference apparatus was used to measure the threshold concentration(s) of Krenite which elicited either of these behavioural responses.

Results varied depending on the life stage or the physico-chemical characteristics of the diluent water which was tested. The significance of evaluating environmental variables and mechanisms of effect is discussed.

PROCÉDURES EN LABORATOIRE POUR ÉVALUER LES EFFETS AIGUS ET CHRONIQUES CAUSÉS PAR L'HERBICIDE KRÉNITE (UTILISÉ DANS LE CONTRÔLE DES BROUISSAILLES) SUR LES SALMONIDES

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On a eu recours à plusieurs tests à toxicité létale et sous-létale pour déterminer l'ampleur potentielle de la menance portée par la pulvérisation aérienne du Krenite (phosphonate d'ammonium éthyle carbamyle, Dupont Canada), un herbicide utilisé dans le contrôle des broussailles afin de faciliter la pousse des conifères, sur les salmonidés résidents et migrateurs. On développa des bioassais en laboratoire dans le but d'examiner l'influence de différentes conditions environnementales (dont la qualité des eaux tributaires) sur la tolérance de ces poissons au Krenite, et pour déterminer les effets adverses immédiats et à long terme occasionnés par différentes doses sur certaines étapes de leur cycle de vie. Dans le cours de cette étude, on détermina aussi les concentrations d'herbicide critiques produisant des réactions de stress et des réponses de répulsion/attraction chez les salmonidés juvéniles.

On a déterminé la tolérance relative au Krenite de huit étapes du cycle de vie du saumon coho (oeufs récemment fertilisés jusqu'à tacon) et de cinq étapes du cycle de la truite arc-en-ciel (oeufs récemment fertilisés à alevins). Cette tolérance fut déterminée par des bioassais de concentration létale médiane (CL_{50}) de 4 et 12 jours. Les groupes de poissons ayant survécu à l'exposition à des concentrations diluées de Krenite au début de leur cycle vital, furent élevés jusqu'au stade d'alevin dans de l'eau douce non contaminée. On nota les paramètres suivants pour ces poissons: taux de survie après exposition, temps jusqu'à éclosion, taux de succès d'éclosion, temps d'absorption du vitellus, croissance, anomalies comportementales et développementales. Durant un deuxième essai, on détermina leur tolérance au Krenite ou à une substance toxique de référence, le pentachlorophénol. On examina le taux de mortalité en eau douce et en eau salée des saumons coho pré-tacons et tacons ayant survécu à l'exposition à court terme au Krenite.

Des études à exposition contrôlée furent conduites avec des truites arc-en-ciel et des saumons coho de moins d'un an dans le but de déterminer l'influence de certaines variables environnementales sur leur tolérance létale aiguë au Krenite. Ces études comprenaient l'examen de l'influence des caractéristiques de l'eau diluente, du pH, des températures d'analyse et d'acclimatation sur les valeurs du CL_{50}. On détermina aussi les effets du vieillissement du Krenite en eau douce, de la densité des poissons durant les bioassais, et des bioassais statiques vs. ceux à remplacement sur les valeurs CL_{50}.

On détermina la concentration médiane effective (CE_{50}) du Krenite, qui cause un déclin dans le nombre de leucocytes en circulation (mesurée en termes de valeurs de leucocrite) et dans le nombre d'érythrocytes en circulation (mesuré en termes d'hématocrite) chez l'alevin de saumon coho. Ces données furent interprétées comme étant la concentration de Krenite qui est extrêmement

dommageable à cette étape du cycle de vie du salmonidé. Un appareil se servant du gradient des réponses d'évitement/préférence fut utilisé pour mesurer les concentrations de Krenite élicitant chacun de ces comportements.

Les résultats varièrent selon l'étape du cycle vital ou selon les caractéristiques physico-chimiques de l'eau de dilution étant testée. L'importance de l'évaluation des variables et des mécanismes environnementaux relevants est discutée.

INDUCIBLE ENZYMES: POTENTIAL DIAGNOSTIC AIDS IN CASES OF SUSPECTED CHEMICAL POISONING OF MARINE MAMMALS

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The induction of liver enzymes following exposure to certain chemicals has been reported in a number of vertebrate taxa from fish to mammals. There is little literature to describe the response in marine mammals, but analysis of dead marine mammals frequently reveals the presence of numerous chemicals. Among the chemicals with inducing properties in other vertebrates are pollutants like certain PCB's, petroleum oils and polyaromatic hydrocarbons. We have examined three liver enzymes in young non-captive harp seals injected experimentally with Norman Wells crude oil and then sacrificed at intervals after the treatment. Relative to untreated seals of the same age the injected animals had clear elevations in all three liver enzymes examined, namely aryl hydrocarbon hydroxylase (benzo(a)pyrene as substrate), o-demethylase (p-nitroanisole substrate), and o-deethylase (7-ethoxyresorufin substrate). Study of the enzyme activity in microsomal preparations from stored frozen liver showed that treated and untreated seals were as readily distinguished then as they were when analyzed on the day of collection. Furthermore, fresh liver tissue was stored on ice at 0°C and analyzed repeatedly over 10 days for o-deethylase activity, and there was very little loss of activity over the period. Given the responsiveness of the enzymes in harp seal pups, and their apparent storage stability, they seem to offer potential as screening tools in the pathology of marine mammals to support or to refute hypotheses linking injury or death of these animals with exposure to chemical pollutants.

ENZYMES INDUITS: AIDE POTENTIELLE POUR LE DIAGNOSTIQUE DES CAS OÙ UN EMPOISONNEMENT D'ORIGINE CHIMIQUE EST SUSPECTÉ CHEZ DES MAMMIFÈRES MARINS

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L'induction d'enzymes hépatiques conséquente à l'exposition à certains produits chimiques a été reportée dans plusieurs taxons de vertébrés, allant des poissons aux mammifères. La littérature pertinente à la réaction ayant lieu dans les mammifères marins est minimale, mais l'étude de mammifères marins morts révèle souvent la présence de nombreux composés chimiques. Parmi les composés chimiques avec des propriétés d'induction dans d'autres vertébrés, on retrouve des agents polluants comme certains BPCx (PCBs), des huiles de pétrole et des hydrocarbures polychromatiques. Nous avons examiné trois enzymes du foie de jeunes phoques du Groenland en liberté auxquels on avait injecté expérimentalement du pétrole brut Norman Wells. Ces animaux furent ensuite sacrifiés à certains intervalles après le traitement. Comparés à des phoques de même âge non-traités, les animaux injectés avaient des niveaux clairement plus élevés des trois enzymes hépatiques examinés. Ces derniers sont l'hydroxylase d'hydrocarbone aryle (benzo(a)pyrène comme substrat), la o-déméthylase (p-nitroanisole comme substrat) et l'o-dééthylase (7-éthoxyrésorufine comme substrat). L'étude de l'activité des enzymes dans les préparations microsomales de foie congelé a montré que les phoques traités et non-traités sont aussi faciles à distinguer après avoir été congelés que quand ils sont analysés le jour même de la collection. De plus, des tissus de foie frais furent entreposés sur la glace à 0°C et analysés à maintes reprises en 10 jours pour étudier l'activité de l'o-dééthylase, et il n'y eut que peu de perte d'activité dans cette période. Etant donné la réponse des enzymes dans les chiots de phoque du Groenland et leur apparente stabilité durant l'entreposage, ils apparaissent prometteurs comme outils de dépistage dans la pathologie des mamifères marins, en vue de supporter ou de réfuter les hypothèses liant les blessures ou la mort de ces animaux à l'exposition aux agents polluants chimiques.

GROWTH AND FEEDING BEHAVIOR RESPONSES OF LARGEMOUTH BASS (MICROPTERUS SALMOIDES) EXPOSED TO PCP

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We monitored food conversion efficiency (g of growth/g of food consumed) of individually housed largemouth bass (Micropterus salmoides) exposed to PCP in two 7-day experiments. The first experiment considered simultaneously the effects of two factors: ration level and PCP level. We found that at both high and low ration levels food conversion efficiencies were reduced 30% (relative to the respective untreated groups) by exposure to 50 μg PCP/L. Feeding behaviour (indicated by capture/strike ratio, and food consumption) was reduced by PCP at the high ration level, but not the low ration level.

In the second experiment we compared the food conversion efficiency of bass exposed to a variety of PCP concentrations. We found a threshold for food conversion efficiency at approximately 25 μg PCP/L. The food conversion efficiency response was the most sensitive measure of the toxicity tests carried out in our laboratory (cf. behaviour, mortality over 120 days, etc.).

The design of both these experiments provided a sensitive measure of toxicity over a 7-day experiment as individual housing of the fish allowed monitoring of weight change and food consumption for each fish. These results are included in manuscripts which will be published in the near future.

A. Mathers, P.H. Johansen, and J.A. Brown. Growth and feeding behavior responses of largemouth bass (Micropterus salmoides) exposed to PCP.

LES RÉACTIONS D'ALIMENTATION ET DE CROISSANCE D'ACHIGANS À GRANDE BOUCHE (*MICROPTERUS SALMOIDES*) EXPOSÉS AU PCP

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Nous avons suivi l'efficacité de conversion de la nourriture (g de croissance/g de nourriture) consommée par des achigans à grande bouche (*Micropterus salmoides*) gardés individuellement et exposés au PCP dans deux expériences de 7 jours chacune. La première expérience considérait simultanément les effets de deux facteurs: la grosseur des rations et le niveau de PCP. Nous avons trouvé qu'aux deux niveaux de ration, grosse et petite, l'efficacité de conversion de la nourriture est réduite de 30% (relativement au groupe respectif non traité) par l'exposition à 50 µg de PCP/L. Le comportement d'alimentation (indiqué par le rapport capture/attaque, et par la consommation de la nourriture) fut réduit par le PCP à haut niveau de ration, mais non pas à bas niveau de ration.

Dans la deuxième expérience, nous avons comparé l'efficacité de conversion de nourriture d'achigans exposés à plusieurs concentrations différentes de PCP. Nous avons trouvé un seuil d'efficacité de conversion de nourriture à environ 25 µg de PCP/L. La réponse d'efficacité de conversion de nourriture était la mesure la plus sensible parmi les tests de toxicité menés dans notre laboratoire (cf. comportement, mortalité dans 120 jours, etc ...).

La conception de ces deux expériences donne une mesure de toxicité sensible pour une expérience durant 7 jours, étant donné que le logement individuel des poissons permet de suivre le changement de poids et la consommation de nourriture pour chaque poisson. Ces résultats sont inclus dans des manuscrits qui devraient être publiés dans un proche futur.

THE SEASONAL EFFECT OF SUB-LETHAL PENTACHLOROPHENOL EXPOSURE ON OVARIAN DEVELOPMENT IN RAINBOW TROUT (SALMO GAIRDNERI)

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The sub-lethal effect of pentachlorophenol (PCP), a widely used pesticide in the forest products industry, on ovarian development has not been examined in fish. During the annual development the female rainbow trout ovary passes through three primary stages: [1] a rapid RNA synthesizing protein production stage, [2] a primary yolk deposition stage and [3] a secondary yolk deposition stage. Dependent on the time of year these major oocytes stages are evident in the ovaries of maturing female rainbow trout. In this study five histological categories were described for oocyte classification. The effect of sub-lethal exposure to PCP on the presence and development of these successive oocyte stages throughout the year was studied.

Rainbow trout acclimated to 12°C were held under a 12-hour light-dark photoperiod and toxicant exposed for 18 days. All fish were sampled and the ovaries removed from females for quantitative histological analysis. Experiments conducted in early winter (December) and summer (July) utilized three different concentrations of PCP: 18, 24 and 51 µg/L and 13, 25 and 50 µg/L respectively. A significant delay effect on the development of the respective oocyte stages present was determined. A dose-response on stage succession with increasing PCP concentration was also noted. Studies involving just one level of PCP (25 µg/L) in spring (March) and autumn (October) also produced the delay effect.

These results are discussed as to the effect on any predominant oocyte stage as they arise during the annual cycle. Some particularly sensitive periods with respect to ovarian maturation will be emphasized.

L'EFFET SAISONNIER DE L'EXPOSITION SUB-LÉTALE AU PENTACHLOROPHÉNOL SUR LE DÉVELOPPEMENT DES OVAIRES DANS LA TRUITE ARC-EN-CIEL (SALMO GAIRDNERI)

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L'effet sub-létal du pentachlorophénol (PCP), un pesticide largement utilisé par l'industrie forestière, n'a jamais été étudié sur le développement ovarien des poissons. L'ovaire des truites arc-en-ciel femelles passe par trois étapes durant son développement annuel: [1] une étape rapide de production de protéines synthétisantes d'ARN, [2] une étape de déposition de jaune d'oeuf primaire, et [3] une étape de déposition de jaune d'oeuf secondaire. Selon le temps de l'année, ces majeures étapes d'oocyte sont évidentes dans les ovaires des truites arc-en-ciel femelles et matures. Dans cette étude, cinq catégories histologiques furent décrites pour la classification des oocytes. On étudia l'effet de l'exposition sub-létale au PCP, à l'année longue, sur la présence et sur le développement de ces étapes d'oocyte consécutives.

Des truites arc-en-ciel acclimatées à 12°C furent gardées sous un régime de photopériode de 12 heures (lumière et noirceur) et furent exposées au produit toxique pour 18 jours. Tous les poissons furent échantillonnés et leurs ovaires furent enlevés pour des analyses histologiques quantitatives. Les expériences conduites au début de l'hiver (décembre) et durant l'été (juillet) utilisèrent trois concentrations différentes de PCP: 18, 24 et 51 μg/L et 13, 25 et 50 μg/L respectivement. On détermina un effet de retardement important sur le développement des étapes d'oocyte. On nota aussi un effet sur la succession des étapes d'oocyte qui variait selon le dosage et avec l'augmentation dans la concentration de PCP. Des études impliquant seulement un niveau de PCP (25 μg/L) au printemps (mars) et en automne (octobre) produisirent aussi un effet de retardement.

Ces résultats sont discutés par rapport à l'effet produit sur n'importe quelle étape d'oocyte prédominante à un certain moment du cycle annuel. On mettra l'accent sur certaines périodes particulièrement sensibles par rapport à la maturation de l'ovaire.

STREAM CHANNEL EXPERIMENTS ON BEHAVIOURAL RESPONSES OF ACRONEURIA LYCORIAS (INS., PLECOPT.) TO METHOXYCHLOR, FENITROTHION AND LOWERED PH

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Laboratory stream channels were used to investigate responses of Acroneuria lycorias (Ins., Plecopt.) to three different stressors: methoxychlor, fenitrothion and lowered pH. Microhabitat preferences under unstressed conditions lead, in accordance with field observations, to distribution patterns determined by thigmotaxis and negative phototaxis. Both methoxychlor and fenitrothion exposure at sublethal concentrations cause abandonment of microhabitats, associated with increased locomotor activity and drift. In the case of the polychlorinated hydrocarbon pesticide methoxychlor, the response is very rapid (starting within 5 min) and sensitive (threshold ≈0.5 to 1.0 μg/L). The organophosphate fenitrothion causes a delayed response (approximately 7-12 h after onset of exposure), with a threshold of 8 to 12 μg/L. Lowering of pH from 8.0 to 2.5 at a rate of ≈0.6 pH units/h led to respiratory distress symptoms at pH 3 and lower, but not to abandonment of microhabitats.

DES EXPÉRIENCES SUR LES RÉPONSES COMPORTEMENTALES D'ACRONEURIA LYCORIAS (INSECTA, PLECOPTERA) AU MÉTHOXYCHLORE, FÉNITROTHION ET LE BAS PH EN RUISSEAU ARTIFICIEL

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On a utilisé des ruisseaux artificiels en laboratoire pour analyser les réponses d'Acroneuria lycorias (Ins., Plecopt.) à trois différentes sources de stress: le méthoxychlore, le fénitrothion et le bas pH. Les préférences pour certains microhabitats sous des conditions non stressantes aboutissent, et ceci en accord avec les observations faites sur le terrain, à des modes de distribution déterminés par le thigmotactisme et par le phototactisme négatif. Des expositions au méthoxychlore et au fénitrothion à des concentrations sub-létales causent l'abandon des microhabitats, couplé à une augmentation des activités locomotrice et de dérive. Dans le cas du pesticide à base d'hydrocarbures polychlorés, méthoxychlore, la réponse est très rapide (commençant en dedans de 5 minutes) et sensible (seuil ≈ 0.5 à 1.0 μg/L). L'organophosphate fénitrothion cause une réponse retardée (approximativement 7 à 12 h après le commencement de l'exposition), avec un seuil de 8 à 12 μg/L. La diminution du pH de 8.0 à 2.5 à une vitesse de ≈ 0.6 unités de pH/h mène à des symptômes d'affliction respiratoire à un pH de 3 et plus bas, mais non à l'abandon des microhabitats.

ASSESSING SALMONID RESPONSE TO GAS SUPERSATURATION WITH A NEW MULTIVARIABLE DOSE-RESPONSE MODEL

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Data on exposure time to 50% mortality (ET_{50} h) from the literature are examined in juvenile salmonids in response to excess total gas pressure (TGP%, % saturation) as influenced by water depth, fish length and water temperature. The method presents [1] a technology for preliminary analysis of such multivariate data with 'tabular histograms', [2] a general model for assessing the influence of environmental factors on fish mortality and [3] an application of these methods to the compiled data. Although the data exhibit gaps in the distributions of some factors (particularly from 100 to 110 TGP%), they are adequate for building preliminary ET_{50} response surface models. The predicted ET_{50} follows a typical exponential dose-response relationship with TGP% in excess of 100%. Furthermore, both water depth and fish length significantly increase ET_{50} (i.e., allow fish to live longer) when either ancillary factor is increased. Temperature has a smaller modifying effect, causing ET_{50} to decrease (i.e., fish to die sooner) at higher temperatures. Models are also developed on the basis of a smaller data set including information on dissolved oxygen (O_2%, % saturation) and nitrogen (N_2%, % saturation). A two-predictor model based on TGP% and either O_2% or O_2%/N_2% ratio shows that reducing O_2%, while holding TGP% constant, decreases ET_{50}. Therefore, no general safe level of TGP% in excess of 100% can be recommended. Instead, a threshold level of excess TGP% may be calculated by setting a minimum time limit to 50% mortality and including values for the ancillary factors modelled. Fifty-day ET_{50} thresholds range from 109.2% to 115.1% saturation, depending on associated values of water depth, fish length and temperature. Furthermore, if only TGP% and the O_2%/N_2% ratio are used to predict ET_{50}, then 50-day TGP% thresholds range from 108.3% to 112.3% saturation at O_2%/N_2% ratios of 0.5 and 1.5, respectively.

L'ESTIMATION DES RÉPONSES DE SALMONIDES À LA SURSATURATION DE GAS AVEC UN NOUVEAU MODÈLE DE RÉPONSE À DOSE MULTIVARIABLE

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Des données sur le temps d'exposition nécessaire pour atteindre 50% de mortalité (TE_{50} h) chez les salmonidés juvéniles, prises à partir de la littérature, sont examinées pour déterminer l'influence de la profondeur, de la température de l'eau et de la longueur des poissons sur leur réponse à des excès de pression gazeuse totale (PGT, % de saturation). La méthode présentée inclut [1] une technologie pour l'analyse préliminaire de telles données multi-variées avec des 'histogrammes tabulaires', [2] un modèle général pour estimer l'influence des facteurs environnementaux sur la mortalité des poissons, et [3] l'application de ces méthodes à des données déjà compilées. Bien que les données aient des 'trous' dans la distribution de certains facteurs (particulièrement de 100 à 110 PGT%), elles sont adéquates pour construire des modèles de surface préliminaires pour les réponses de TE_{50}. Le TE_{50} prédit, suit une courbe de réponse-dose exponentielle typique en relation avec la PGT% en excès de 100%. De plus, la profondeur de l'eau et la longueur des poissons augmentent le TE_{50} significativement (i.e. permettent aux poissons de survivre plus longtemps) quand l'un ou l'autre des facteurs auxiliaires est augmenté. La température a un plus petit effet modificateur, causant une diminution du TE_{50} (i.e. les poissons meurent plus tôt) à de hautes températures. Des modèles sont aussi développés à partir de bases de données plus petites incluant de l'information sur l'oxygène dissous (O_2%, % de saturation) et sur de l'azote dissous (N_2%, % de saturation). Un modèle basé sur une PGT% et sur un des rapports O_2% ou $O_2\%/N_2\%$ montre que la réduction de O_2%, tout en tenant la PGT% constante, diminue le TE_{50}. Par conséquent, il n'existe pas de niveau général de sécurité de PGT% en excès de 100% pouvant être recommandé. Comme alternative, un seuil du niveau d'excès de PGT% peut être calculé en fixant une limite minimum de temps à 50% de mortalité et en incluant les valeurs pour les facteurs auxiliaires modelés. Les seuils de TE_{50} de cinquante jours varient de 109.2% à 115.1% de saturation selon les valeurs de profondeur d'eau, de longueur de poissons et de température. De plus, si on utilise seulement la PGT% et le rapport $O_2\%/N_2\%$ pour prédire le TE_{50}, les seuils de PGT% de 50 jours varient de 108.3% à 112.3% de saturation à des rapports $O_2\%/N_2\%$ de 0.5 et 1.5, respectivement.

ASBESTOS FIBRES AND ASSOCIATED TRACE METAL TOXICITY IN STREAM WATER

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It is well known that airborne exposure to asbestos fibres leads to high cancer incidence but at present the effect of asbestos fibre ingestion via drinking water is less certain. In spite of numerous studies, only the San Francisco Bay project has shown a positive link between asbestos concentration in drinking water and cancer rates. In recent years most medical researchers have dismissed chemicals associated with asbestos as a likely cause of cancer and have instead focused on fibre geometry. However, research on asbestos-rich serpentinitic soils has shown that nutrient imbalances and very high levels of Ni, Cr, Co and Mn associated with asbestos were found to inhibit micro-organisms and plant growth. To document the extent of the problem in the aquatic environment a study was initiated in the Sumas River where a major landslide has exposed a serpentinitic formation which introduces large quantities of asbestos fibres into the stream. During flooding periods asbestos-rich sediments have been deposited in a number of fields downstream causing toxicity problems to the plants and rural population. Our investigation shows that there is a direct relationship between asbestos fibres, trace metal concentrations and stream discharge. In the vicinity of the landslide Cr, Ni and Mn values were consistantly above drinking water standards during peak flow and preliminary work shows that bacterial activity and numbers might be supressed near the point source. Earthworms incubated in the sediments accumulated high quantities of Mn, Ni and Cr and were subject to 100% mortality when the sediments were acidified. It appears that trace metal toxicity might affect the stream biology and an examination of trace metal accumulation in fish tissue is currently underway.

TOXICITÉ DE MÉTAUX À L'ÉTAT DE TRACES ASSOCIÉE AUX FIBRES D'AMIANTE EN EAU DOUCE

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C'est un fait reconnu que l'exposition aux fibres d'amiante dans l'air mène à de hautes fréquences de cancer, mais à présent l'effet d'ingestion des fibres d'amiante à travers l'eau potable est moins certain. En dépit de plusieurs études, le seul projet à avoir démontré un lien positif entre les concentrations d'amiante dans l'eau potable et le taux de cancer est celui de la baie de San Francisco. Dans les dernières années, la plupart des chercheurs médicaux ont rejeté les produits chimiques associés à l'amiante comme cause probable de cancer et se sont concentrés sur la géométrie des fibres. Cependant, des recherches sur les terres serpentinitiques, riches en amiante, ont révélé des déséquilibres dans les éléments nutritifs et ont montré que les très hauts niveaux de Ni, Cr, Co et Mn associés avec l'amiante inhibent la croissance des micro-organismes et des plantes. Pour documenter l'étendue du problème dans l'environnement aquatique, on a commencé une étude dans la rivière Sumas où un glissement de terrain majeur a exposé une formation serpentinitique qui introduit de grandes quantités de fibres d'amiante dans la rivière. Durant les périodes d'inondation, les sédiments riches en amiante se sont déposés dans plusieurs champs en aval, causant des problèmes de toxicité aux plantes et à la population rurale. Notre recherche démontre qu'il y a une relation directe entre les fibres d'amiante, les concentrations de métaux à l'état de traces et le volume de décharge de la rivière. Dans les environs immédiats du glissement de terrain, les valeurs de Cr, Ni et Mn sont régulièrement au-dessus des standards d'eau potable durant le maximum d'écoulement et les travaux préliminaires montrent que l'activité et le nombre de bactéries peuvent être supprimés près du point de source. Des vers de terre incubés dans les sédiments accumulèrent de hautes quantités de Mn, Ni et Cr et furent sujets à une mortalité de 100% quand les sédiments furent acidifiés. Il semble que la toxicité des métaux à l'état de traces puisse affecter la biologie des cours d'eau et un examen de l'accumulation des métaux à l'état de traces dans les tissus de poissons est présentement en cours.

ECOTOXICOLOGICAL EVALUATION OF LAKE ST. LOUIS (ST. LAWRENCE RIVER) SEDIMENTS

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Lake St. Louis is formed by the confluence of the Ottawa and St. Lawrence Rivers near Montreal. It receives toxic chemicals from diffuse sources, such as Lake Ontario (e.g. PCB, mirex) and the Ottawa River (Hg). There are, however, important local sources, of which the most important is probably a chlor-alkali plant (Hg).

To determine the extent and significance of toxic chemicals in Lake St. Louis sediments, as well as the relative contributions of the various sources, an intensive survey was carried out at about 50 stations located throughout the lake. The sediment samples were analysed for chemical and biological parameters: geochemistry, contaminants (heavy metals, Hg, PCB/OC, PAH, chlorobenzenes and chlorophenols), bacteriological parameters (heterotrophic activity, respiration, N- and S-cycle bacteria, ATP biomass) and bioassays on sediment elutriates (Microtox, algal C^{14} uptake, Daphnia, rotifers and nematodes).

This particular ecotoxicological approach to environmental monitoring of toxic chemicals and some of the results are discussed. Special emphasis will be given to the relationship between the presence of toxic chemicals (chemical analyses) and the various toxic responses observed (biological analyses).

ÉVALUATION ÉCOTOXICOLOGIQUE DES SÉDIMENTS DU LAC ST-LOUIS (FLEUVE ST-LAURENT)

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Le lac St-Louis est formé à la confluence de la rivière Ottawa et du fleuve St-Laurent près de Montréal. Il reçoit des produits toxiques provenant de sources variées, telles le lac Ontario (e.g. PCB, mirex) et la rivière Ottawa (Hg). Il y a cependant d'importantes sources locales, dont la plus importante est probablement une usine de chlore-alcali (Hg).

On a mené une étude intensive à environ 50 stations éparpillées dans le lac St-Louis en vue de déterminer l'étendue et l'importance des produits chimiques dans les sédiments du lac, de même que la contribution relative des sources de pollution. Les échantillons de sédiments furent analysés pour les paramètres chimiques et biologiques: géochimie, contaminants (métaux lourds, Hg, PCP/OC, HAP, chlorobenzènes et chlorophénols), paramètres bactériologiques (activité hétérotrophique, respiration, bactéries de cycle -N et -S, biomasse d'ATP) et bioassais sur les sédiments séparés par décantation (Microtox, absorption de C^{14} par les algues, Daphnia, rotifères et nématodes).

On discutera cette approche écotoxicologique de la surveillance environnementale des produits chimiques toxiques de même que quelques résultats. On portera une attention particulière à la relation entre la présence de produits chimiques toxiques (analyses chimiques) et les réponses toxiques observées (analyses biologiques).

DEVELOPMENT AND TESTING OF A STANDARDIZED AQUATIC MICROCOSM PROTOCOL: STATUS

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The hypothesis that standardization of initial chemical and biological conditions will yield a microcosm bioassay that provides similar responses to toxicants in different laboratories is being tested. The microcosms consist of 3 L of chemically defined freshwater-medium, sediment, 10 algal and 5 animal species (Daphnia, amphipods, ostracods, rotifers and protozoa). Experiments involve 24 microcosms, consisting of 4 treatment groups of 6 replicates each. Test chemicals are added on day 7 and species abundances and chemical variables are monitored once or twice per week through day 63. The data processing, graphics and statistical analyses are computerized.

Microcosm responses to the test chemical, $CuSO_4$, will be compared to responses observed in natural communities including CEPEX experiments. Results support the hypothesis that grazer-controlled communities display delayed algal blooms if grazers are more sensitive and have slower recovery times than Cu-tolerant algae. Some, but not all natural communities have these properties.

Analyses of the repeatability of results within our laboratory and reproducibility of results in other laboratories is a major portion of the current work. If results demonstrate that it is possible to obtain consistent responses to a toxicant in different laboratories, it will increase the acceptance of multi-species data for the testing of the environmental safety of new chemicals which are likely to be released in a wide variety of environments. Hopefully, the research will contribute to our understanding of how changes in community structure or controlling factors modify responses to chemical stress. This would aid in extrapolating results between communities.

DÉVELOPPEMENT ET TEST D'UN PROTOCOLE STANDARD DE MICROCOSME AQUATIQUE: OÙ EN EST-ON?

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On est en train de tester l'hypothèse que la standardisation des conditions chimiques et biologiques initiales produira un bioassai de microcosme qui fournira des réponses similaires aux substances toxiques dans des laboratoires différents. Les microcosmes sont constitués de 3 L d'eau douce à propriétés chimiques définies, de sédiments et de 10 espèces d'algues et 5 d'animaux (Daphnia, amphipodes, ostracodes, rotifères et protozoaires). Les expériences comprennent 4 groupes de traitements de 6 copies chacun. Les produits chimiques testés sont additionnés au 7ème jour et le nombre d'espèces ainsi que les variables chimiques sont relevées une à deux fois par semaine durant 63 jours. Le traitement des données et les analyses graphiques et statistiques sont faits par ordinateur.

Les réponses du microcosme au produit chimique testé, le $CuSO_4$, seront comparées aux réponses observées dans les communautés naturelles comprenant des expériences CEPEX. Les résultats supportent l'hypothèse que les communautés contrôlées par les brouteurs ont leurs explosions de populations d'algues retardées si les brouteurs sont plus sensibles et ont un temps de récupération plus lent que les algues tolérantes au Cu. Certaines, mais non pas toutes les communautés naturelles ont ces propriétés.

Les analyses de répétabilité des résultats conduites dans notre laboratoire et les résultats de répétabilité d'autres laboratoires constituent une majeure portion de notre travail actuel. Si ces résultats démontrent qu'il est possible d'obtenir des réponses uniformes à un produit toxique dans différents laboratoires, ceci augmentera l'approbation des résultats compilés à partir de plusieurs espèces pour les analyses regardant le danger potentiel porté à l'environnement par de nouveaux produits chimiques risquant d'être déversés dans une multitude de milieux. Nous espérons que cette recherche contribuera à la compréhension du rôle que la structure des communautés ou des facteurs de contrôle jouent dans la modification des réponses au stress chimique. Ceci devrait aider dans l'extrapolation des résultats entre communautés.

FROM DATA COLLECTION TO INTERPRETATION: WHAT DOES IT ALL MEAN?

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Collection, handling, statistical analyses and interpretation of data from aquatic microcosms has much in common with that of field studies. Although use of a standardized microcosm has made data collection and handling easier, its interpretation and display of meaningful results remains complex.

Computer-generated tables and graphics of replicate data, probabilities of treatment effects, and verbal interpretations of selected data will be displayed. Meeting participants will be asked to indicate their preference for alternate display forms. When does too much information overwhelm rather than inform?

Statistical properties of ecosystem data pose common problems. Data are often collected sequentially on related variables thus violating statistical test assumptions of independence (e.g. sequences of pH, chlorophyll, algal cell numbers, grazers). Some data sets of ecological variables display properties that make it difficult to display statistical differences within the range of biologically possible responses; in such cases, what is the meaning of the statement, "No statistical differences were displayed."?

What are appropriate end points of ecosystem bioassays? For example, does the substitution of one algal species by another with similar availability and food value to grazers constitute a major ecological change? Given large numbers of statistical comparisons, how does the researcher distinguish between "random" distribution of statistical difference and biologically meaningful ones? Chemical variables usually display less variance than population estimates and therefore display more statistical differences between treatments; are chemical data therefore more important? Can the evaluation of ecosystem data be automated in an objective fashion, or will it remain largely subjective?

Data and statistical analyses displaying such properties will be displayed along with suggestions of operational interpretations. The authors hope to initiate discussion and correspondence on these problems.

DE LA COLLECTION DES DONNÉES À LEUR INTERPRÉTATION: QU'EST-CE QUE CA VEUT DIRE?

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La collection, la manipulation, l'analyse statistique et l'interprétation de données provenant de microcosmes aquatiques ont beaucoup en commun avec les opérations similaires faites sur le terrain. Bien que l'utilisation d'un microcosme standardisé a rendu plus facile la collection et la manipulation des données, leur interprétation et la présentation de résultats éloquents demeurent complexes.

Nous présenterons des tableaux et graphes de données dupliquées, faits par ordinateur, des probabilités d'effets de traitements et des interprétations verbales de certaines données. Nous demanderons aux gens présents d'indiquer leurs préférences par rapport à différentes formes de présentation de données. A quel point un surplus d'information devient-il écrasant?

Les propriétés statistiques de données provenant d'écosystèmes posent des problèmes communs. Des données prises sur des variables apparentées sont souvent collectées en séquences, ce qui viole les suppositions d'indépendance des tests statistiques (e.g. des séquences de pH, de chlorophylle, des comptes de cellules d'algues, de brouteurs). Certains groupes de données sur des variables écologiques ont des propriétés qui rendent difficiles la mise en évidence de différences statistiques à l'intérieur des limites imposées par la gamme des réponses biologiques possibles; dans de tels cas quelle est la signification de la phrase "Il n'y avait aucune différence statistique"?

Quels sont les aboutissements pertinents pour des essais biologiques performés sur des écosystèmes? Par exemple, est-ce que la substitution d'une espèce d'algue par une autre qui a une abondance similaire et une valeur nutritive équivalente pour les brouteurs constitue un changement écologique majeur? Vu le grand nombre de comparaisons statistiques, comment le chercheur peut-il distinguer entre des différentes statistiques distribuées 'au hasard' et d'autres biologiquement importantes? Les variables chimiques ont d'habitude une plus petite variance que les estimés de populations et montrent par conséquent plus de différences statistiques entre les traitements; les données sur des produits chimiques sont-elles par conséquent plus importantes? Est-ce que l'évaluation de données provenant d'écosystèmes peut être automatisée d'une façon objective, ou est-elle condamnée à demeurer en grande partie subjective?

Des données et des analyses statistiques ayant de telles caractéristiques seront présentées, de même que des suggestions pour des interprétations opérationnelles. Les auteurs espèrent générer une discussion et initier une correspondance sur ces problèmes.

ADENYLATE ENERGY CHARGE - A POSSIBLE INDICATOR FOR PHYTOPLANKTON UNDER ENVIRONMENTAL STRESS

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Adenylate energy charge (AEC) is an indicator of the metabolic energy state of an organism and is the ratio of the concentrations of various adenine nucleotides. The relationship is expressed by the following:

$$\mathrm{AEC} = \frac{\mathrm{ATP} + 0.5\ \mathrm{ADP}}{\mathrm{ATP} + \mathrm{ADP} + \mathrm{AMP}}$$

Any factor that upsets the well-being of an organism will be reflected in changes in AEC. Generally, AEC values above 0.7 indicate the organism is in a non-stressful environment and values below 0.7 are indicative of stress.

The AEC values have been used quite successfully in measuring the responses of fish and invertebrates to environmental perturbation. Not much is known about the application of these values to phytoplankton. We have improved the method for AEC analyses in phytoplankton and have measured the AEC values in phytoplankton under chemical and physical stress. We have compared the technique with the conventional ^{14}C-primary productivity method. We are currently analyzing the AEC values in phytoplankton from several areas known to be contaminated with pollutants.

LA CHARGE ÉNERGÉTIQUE D'ADENYLATE - UN INDICATEUR POSSIBLE DE PHYTOPLANCTON SOUS STRESS ENVIRONNEMENTAL

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La charge énergétique d'adénylate (CEA) est indicatrice de la condition de l'énergie métabolique d'un organisme et est obtenue par le rapport de concentrations de certains nucléotides d'adénine. La relation peut s'exprimer comme:

$$\text{CEA} = \frac{\text{ATP} + 0.5\ \text{ADP}}{\text{ATP} + \text{ADP} + \text{AMP}}$$

Tout facteur affectant le bien-être d'un organisme se traduira par des changements dans la CEA. En général, des valeurs de CEA au-dessus de 0.7 indiquent que l'organisme est dans un milieu non stressant et des valeurs en-dessous de 0.7 sont indicatives de stress.

Les valeurs de CEA ont été utilisées avec un certain succès pour mesurer la réponse de poissons et d'invertébrés à des perturbations environnementales. On ne connaît que peu de choses quant à l'application de ces valeurs au phytoplancton. Nous avons amélioré la méthode d'analyse de CEA dans le phytoplancton et nous avons mesuré les valeurs de CEA de phytoplancton placé sous stress physique et chimique. Nous avons comparé cette technique avec la méthode conventionnelle de production primaire ^{14}C. Nous sommes présentement en train d'analyser les valeurs de CEA dans du phytoplancton provenant de plusieurs régions reconnues comme étant contaminées par des agents polluants.

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